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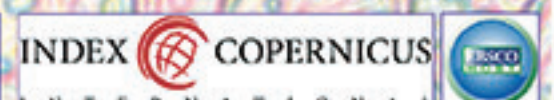

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Impaired movements in 6-OHDA induced Parkinson's rat model improves by pomegranate seed hydroalcoholic extract

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Abstract

Background: The aim of this study was evaluation the effect of PGSE on motor disorders in rats with Parkinson's disease (PD) induced by neurotoxin 6-hydroxy dopamine (6-OHDA). Our Previous findings showed that pomegranate seed hydroalcoholic extract (PGSE) is an excellent natural substance with potent antioxidant effect and free radicals scavenger. PD is clinically characterized by development of motor disturbances, such as bradykinesia, resting tremors, rigidity, and a later loss of postural reflexes. Oxidative stress is a hallmark factor where the oxidation of dopamine generates reactive oxygen species (ROS) and an unbalanced production ROS induces neuronal damage, therefore leading the neuronal death.

Methods: Wistar male rats divided into seven groups randomly with 10 in each. Animals in all groups except sham operated (Sh-PD) and positive control (Cont-P200) groups received 8µg/2µl 6-OHDA dissolved in normal saline contains 0.01% ascorbate or vehicle in right medial forebrain bundle (MFB) under stereotaxic surgery. Two weeks later PD was approved by contralateral rotation sign induced by apomorphine. PD animals received different doses of PGSE (0, 100, 200, 400, 800 mg/kg/2ml, po) or same volume of vehicle for two weeks and Cont-P200 received best effective dose of PGSE. Motor activities were evaluated with standard behavioral tests.

Results: Motor functions were impaired and all doses of PGSE could improve motor dysfunctions in PD rats significantly.

Conclusion: Our results showed that PGSE may act as a potent antioxidant and free radical scavenger to reverse motor disorders after 6-OHDA neurotoxicity in brain.

Key words: Pomegranate, Parkinson's disease, 6- hydroxydopamine, motor functions, rat.

Introduction

Parkinson's disease (PD) is a neurodegenerative disease whose pathogenesis is well understood: The progressive loss of dopamine neurons in the substantia nigra, a nucleus of the midbrain [1]. This cell loss causes a spectrum of movement disorders, including the clinical triad of resting tremor, rigidity and bradykinesia [2-3]. The motor symptoms of Parkinson's disease result from the death of dopamine-generating cells in the substantia nigra, a region of the midbrain, the cause of this cell death is unknown [4]. The most obvious symptoms are movement-related; these include shaking, rigidity, slowness of movement and difficulty with walking and gait. Later, cognitive and behavioral problems may arise, with dementia commonly occurring in the advanced stages of the disease. Other symptoms include sensory, sleep and emotional problems. PD is more common in the elderly, with most cases occurring after the age of 50 [5].

The unilateral injection of 6-hydroxydopamine (6-OHDA) into medial forebrain bundle (MFB) of rats is frequently used to making an animal model of Parkinson's disease (PD). MFB lesion model mimics an early stage of PD [6-7]. PD is characterized by the bilateral degeneration of the midbrain dopamine-containing neurons with the most severe lesion in the posterior-lateral part of the substantia nigra pars compacta (SNpc). In humans, such lesions lead to specific motor abnormalities (i.e., akinesia, rigid-

ity, and tremor) that are greatly improved by drug treatment [8]. The 6-OHDA model of Parkinson's disease in the rat represents a fundamental tool for investigating the pathophysiology of dopamine denervation [9]. Since 6-OHDA injections into the MFB and the striatum (STR) result in complete and partial SNc lesions, respectively, it is believed that communication links exist between neurons, along neuronal pathways that transmit activating signals in response to neuronal damage [10].

Free radical formation and oxidative stress might play an important role in the pathogenesis of Parkinson's disease (PD). The central nervous system shows an exceptionally high degree of vulnerability to reactive oxygen species (ROS) [11]. On the other hand, aging is a major risk factor for neurodegenerative diseases including PD, Alzheimer's disease (AD). An unbalanced overproduction of ROS may give rise to oxidative stress which can induce neuronal damage, ultimately leading to neuronal death by apoptosis or necrosis. Numerous evidences indicate that oxidative stress is involved in the pathogenesis of PD and AD [12]. In almost all of these processes, oxidative stress is a hall mark factor where the oxidation of dopamine (DA) generates reactive oxygen species (ROS) and an unbalanced production of ROS induces neuronal damage therefore leading to neuronal death [13-14].

Oxidative stress is related to the production by all aerobic organisms of reactive oxygen and nitrogen species including free radicals and antioxidants in dietary plants [15]. Epidemiological studies suggest that a reduced risk of cancer is associated with higher consumption of a phytochemical-rich diet that includes fruits and vegetables [16]. Consumption of polyphenoles and flavonoids is benefitial for the perevention of cardiovascular, inflammatory and other diseases [17] by preventing oxidative stress that is lipid peroxidation in arterial macrophage and in lipoproteins [18]. The presence of antioxidants has been reported from pomegranate juice [17, 19].

Pomegranate (Punicagranatum L.), Lythraceae, is mainly grown in Mediterranean regions and is one of the major cultivated productions of Iran, as far north as the Himalayas, in Southeast Asia, and in California and Arizona in the United States. It has been consumed for many centuries or perhaps millenniums as fruit, beverage and food-related product. Pomegranate has been used in Iranian traditional medicine for different therapies. For example, the fruit was effective as diuretic and prokinetic agent and also as liver revival. Some other parts of pomegranate tree were also used in antiparasite and anti-diarrhea formulations. Today pomegranate is known as antimicrobial [20-21], antiviral [22-23], and anticancer [24-25] substance which has led to being the center of attention in many studies. Both pomegranate pulp and peel contain different kinds of antioxidants [26], including those which have not possibly been well characterized so far. It has been acknowledged that phenol compounds such as flavonoids and anthocyanins are the major class of effective antioxidants in many fruits and vegetables [23].

In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-infammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity [19, 27]. Pomegranate contains some species of flavonoids and anthocyanidins in its seed oil and juice and shows antioxidant activity three times greater than green tea extract. Pomegrante juice contains tannins, ellagictannis, anthocyanins, catechins, gallic and ellagic acid as antioxidant chemicals. Pomegrante seeds are known to contain esterogenic compounds [28-29]. Pomegranate fruit extracts (PFEs) possess polyphenolic and other compounds with anti proliferative, pro-apoptotic and anti-inflammatory effects in prostate, lung, and other cancers. Because nuclear transcription factor-kB (NF-kB) is known to regulate cell survival, proliferation, tumorigenesis, and inflammation, it was postulated that PFEs may exert anticancer effects at least in part by modulating NF-kB activity [30]. The predominant organic acid was citric acid followed by malic acid. The peel fraction had the highest total hydrolyzable tannins

content (4792.3-6894.8 mg/100 g of FW). Overall, the highest antioxidant capacity was found in leaves followed by peel, pulp, and seed. Pomegranate seed had an average lipid content of 19.2% with punic acid as the predominant fatty acid. Pomegranate seed had high contents of alpha-tocopherol (161.2-170.1 mg/100 g) and gamma-tocopherol (80.2-92.8 mg/100 g) [31].

A recent review reported the chemical constituents of diverse parts of P. granatum as well as their potential for prevention and treatment of inflammation and cancer. The authors refer that in pericarp, leaf and flower can be detected phenols (flavonoids and tannins) being some of them unique. Complex polysaccharides have also been detected and characterized in the peels. In seeds, triacylglycerols constituted the oil, with a high content of punicic acid. In this oil, the authors also reported the presence of sterols, steroids and cerebroside in very small amounts. In addition to the seed oil, lignin and their derivatives have also been reported possessing remarkable antioxidant activities [27].

Till date the cure for PD is obscure. Many treatments are available which can slow down its progression and most of them can only alleviate the symptoms [32]. Therefore, the best strategy is to prevent the onset of PD. Among the possible preventive strategies, antioxidants supplements, nutritional bioenergetics approaches to enhance mitochondrial function and reduce oxidative damage appear promising [33-34]. Earlier from our laboratory, we have reported that some herbal drugs like Grape seed extract [35], soy extract [36], Gallic acid [37], etc. have shown protection against neurodegenerative disorders such as 6-OHDA induced Parkinson's disease.

So, with consideration of above literatures and our novel findings during experiments, since pomegranate seed hydroalcoholic extract (PGSE) has interest and significant beneficial effects on damaged brain due to hypoperfusion/ischemia (HI) in rats. In current study we have decided to evaluate the effect of pomegranate seed hydroalcoholic extract on motor disorders in animal model of Parkinson's disease.

Materials and methods

Animals: All animals used for this study were adult, male Wistar rats (300-350 g) purchased from Ahvaz Jundishapur University of Medical Sciences (AJUMS) central animal Lab. (Khouzestan, Ahvaz, Iran). Animals were given a standard rodent diet and water *ad libitum* and kept on a 12:12-hour light-dark cycle with lights on at 0700 hr. All experiments were performed during the lights-on period and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and with approval from the AJUMS Animal Care and Use Committee (AJACUC). All efforts were made to minimize animal stress and to reduce the number of rats used for the experiments described below. Prior to the onset of behavioral testing, all animals were gentle handled for 5 days (daily 5 min). The animals were divided randomly into nine groups of 10 in each: 1) Control (intact), 2) sham operated (Sham-PD), received 2µl normal saline containing 0.01% ascorbic acid into right medial forebrain bundle (MFB); 3) lesioned (PD), received 8 µg/2µl 6-hydroxydopamine (6-OHDA) into right MFB; 4) PD-P100, 5) PD-P200, 6) PD-P400, 7) PD-P800, 8) PD-Veh, 9) Cont-P200. Treated groups includes rats suffering with PD that each group received 100, 200, 400 or 800 mg/kg PGSE, p.o, for two weeks respectively, from 14 days after surgery and PD confirmation with rotation testby apomorphine. The dose 200 mg/kg of GSE was selected as a best effective dose based on the dose response study for administration to positive control group (Cont-P200) [35, 37-38].

Animal model of PD: Medial forebrain bundle (MFB) in the right brain hemisphere was lesioned using the Tadaiesky's (2008) method with some modifications [39]. Briefly, Rats were deeply anesthetized with ketamine/xylazine (90/10 mg/kg, ip.). Stereotaxic surgery was performed using the coordinates in Paxinos and Watson atlas: AP: -4.4 mm, ML: -1.2 mm and DV: -8.2 mm from bregma and skull surface [40] and 8µg/2µl 6-hydroxydopamine HBr (Sigma, USA) dissolved in normal saline with 0.01% ascorbic acid was infused into right MFB using a 10 µl Hamilton syringe with a 26-gauge needle connected to a 30-gauge cannula. Following injection, the cannula was left in place for 5 min before being retracted to allow complete diffusion of

the drug. All animals were treated with i.p. injection of 25 mg/kg desipramine (Exeir Pharmacy Co. Iran) 30 min before surgery, in order to protect noradrenergic terminals depletion by 6-hydroxydopamine (6-OHDA). Sham-operated rats followed the same protocol except for the fact that vehicle was injected instead of 6-OHDA (figure 1).

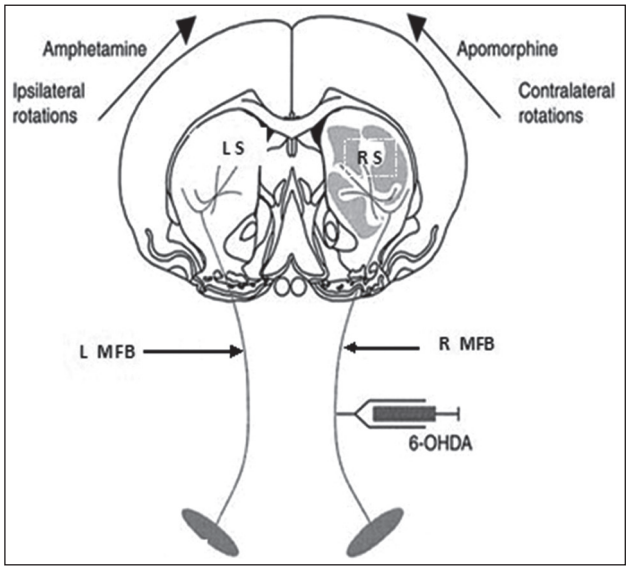

Figure 1. Diagram of the nigrostriatal pathway and rotational responses produced by apomorphine (contralateral rotations) and D-amphetamine (ipsilateral rotations)

Shaded areas in the left striatum indicate the loss of DA due to a MFB injection of 6-OHDA. Abbreviations: RS, right striatum; LS, left striatum; R MFB, right medial forebrain bundle; L MFB, left medial forebrain bundle; 6-OHDA, 6-hydroxydopamine; R SN, right substantia nigra; L SN, left substantia nigra [adapted from [41] with some modification].

PGSE preparation: Pomegranate fruits (*Punica granatum L.*) as large fruit with red barriers produced in Saveh granatum gardens- Iran, were purchased. Seeds removed from the fruits, air dried in shade for one week and milled to fine powder (electric mill, Panasonic Co. Japan). The seeds powder was macerated in 70% ethanol for 72 hours at room temperature. The ethanol extract evaporated (Rotary Ovaporator, Heidolph Co. Germany) to remove ethanol and PGE was obtained as a lyophilized powder (yield 17±2%) [38, 42].

Apomorphine-induced circling behavior: After 14 days of MFB lesioning, the rats were tested for drug-induced rotational behavior just before and after treatment with PGSE. Contralateral rotations of animals were recorded after giving 0.5 mg/kg apomorphine (Sigma, USA, in normal saline containing 0.01% ascorbic acid) subcutaneously to confirm the dopamine depletion in nigrostriatal system. and their rotational scores were collected over a period of 30 min intervals. The animals tested for 5 min over a period of 30 min (6 sessions) for apomorphine rotations. The results were expressed in rotations/10 min [43-44].

Catalepsy tests: The catalepsy was assessed by placing one forepaw on a horizontal bar 9 cm above the surface, and another forepaw on a podium (3 cm high). The latency to initiate the movement was used as a measure of catalepsy. An ability of a tested substance to decrease the latency in the catalepsy test was considered indicative of its potential antiakinetic effect. After then muscle stiffness (rigidity) was tested. The scoring adopted was based on a three stage model as follows: Stage 1; when the rat was placed on a flat table, if it showed normal movement, the score allocated was 0, if the rat did not move, but on gentle touch it showed movement, the allocated score was 0.5. Stage 2; one of the rat forepaws was placed on a 3 cm high wooden podium block, if the rat did not replace its position within 10 s it received a score of 0.5. Similarly, the second forepaw was placed on the wooden block and scored the same. Stage 3; one of the forepaws was placed on a 9 cm high wooden block and another paw left hanging. A positive sign for full rigidity was gauged by the failure of the animal to correct the imposed position within 10 s and was given a score of 1. A similar procedure was used with another forepaw. Thus if a rat was in full rigidity (muscle stiffness), a total cumulative score of 3.5 was assigned [37, 45-46].

Stride length test: Stride length was tested in intact, PD and in PD groups after treatment with PGSE or vehicle. The apparatus was composed of a woody box (20×17×10 cm), in which a runway (4.5-cm wide, 42-cm long with borders of 10-cm height) was arranged to lead out into dark wooden box. Stride lengths were measured by wetting animals' forepaws with commercially available pencil blue or red inks and letting them trot on a strip of paper (4.5-cm wide, 40-cm long) down the brightly lit runway towards the dark goal box. The

forelimb stride lengths were measured for all animals (Control, PD, Sh-PD and PD-P or PD-Veh). Stride lengths were measured manually as the distance between two forepaw prints. The three longest stride lengths (corresponding to maximal velocity) were measured from each run. Paw prints made at the beginning (7 cm) and the end (7 cm) of the run were excluded because of velocity changes. Runs in which the rats made stops or obvious decelerations observed by the experimenter were excluded from analysis [37, 47].

Rotarod (muscular coordination): For motor-coordination ability, 28 days after lesioning, the rats in all groups were evaluated on rotarod apparatus. The rotarod test served the purpose of detecting potential deleterious effects of the compounds studied on the rats' motor performance and coordination. The rotarod unit (Borj Sanaat Co., Tehran, Iran) consist of a rotating rod, 75 mm diameter, on which rats were allowed to retain. The time for each rat to remain on the rotating bar was recorded. The maximum time was 15 minutes per trial. The apparatus automatically records the time in 0.1 s and stop the counting when the rat fall of the rotating shaft. The animals were pre-trained to reach a stable performance in this test. During familiar session, the animals were placed on a rod with constant 5 rpm for 3minutes. Next day during test session, the animals were placed on a rod with an initial constant rod speed of five rotations per minute (5rpm) for 3 minutes, after then speed was increased to 40 rpm programmatically (5-10rpm/next 3 min, 10-20 rpm /next 3 min, 20-30 rpm/ next 3min and 30-40rpm/ end 3min) and cut off time was 15 minutes. The test session consisted of three trials during one day. Inter trial interval was 45 minutes. Data were presented as retention time (seconds) on the rotating bar over the three test trials [37, 45, 48].

Statistics: Data were expressed as mean±S.E.M. of values for motor activity tests. Statistical analysis was performed by Kruskal-waliss followed by post hoc median test for rigidity and by one-way ANOVA followed by LSD post hoc test for other data. A *P-value* less than 0.05 were assumed to denote a significant difference and levels of significance are indicated by symbols: * and # indicated differences between groups vs. Sh-PD and PD groups respectively (* or # $P<0.05$, ** or ## $P<0.01$, and *** or ### $P<0.001$). Because of the control (Cont) and sham operated (Sh-PD) didn't any significant difference so; we used only Sh-PD in figures (except in figure 1) for compare with other groups.

Drugs: Apomorphine and 6-OHDA were purchased from Sigma Chemical Co., USA and Ketamine HCl and xylazine from Alfasan, Woerden–Holland. Desipramine was obtained from Exeir Pharmacy Co., Iran.

Results

Rotation: When the apomorphine was injected subcutaneously into control (intact), sham operated rats and or 14 days after MFB lesioning to PD rats, just after injection rats suffering with PD rotated contralateral to lesioned brain hemisphere significanty with compare to control and Sh-PD groups ($P<0.001$) while rotation was not difference between control and Sh-PD group (figure 2). After 14 days treatment with different doses of PGSE (100, 200, 400, and 800 mg/kg, po) the rotation test was repeated to all groups in order to prove the permanent PD. Contralateral rotation number was decreased in treated rats with PGSE significantly in PD-P200 and PD-P400 ($P<0.05$ and $P<0.01$ for PD-200 and PD-P400 vs. PD group respectively), while it was not in PD-100 and PD-800 groups. These results show that rotational behavior induced by apomorphine in PD rats was permanent after 14 days treatment with lowest and highest doses (100 and 800 mg/kg) of PGSE (figure 3). On the other word, these data showed that rats remained as parkinsonian during total period of experiment.

Bradykinesia: Data have shown that grid descent latency (s) of forepaws on a 9 cm height bar as a valuable parameter for bradykinesia (catalepsy) was increased in PD group significantly (***$P<0.001$) when compared with control or Sh-PD groups, while treatment with PGSE reversed it significantly (***$P<0.001$ for PD, PD-P100 and PD-Veh vs. Sh-PD and ###$P<0.001$ for all PD groups treated with PGSE (PD-P100-800) vs. PD and PD-Veh, figure 4).

Muscle stiffness: Muscle stiffness (rigidity) was increased significantly in PD (***$P<0.001$ for PD vs. Sh-PD groups), while treatment with PGSE decreased it significantly (###$P<0.001$ for PD-P200-800 groups vs. PD, figure 5).

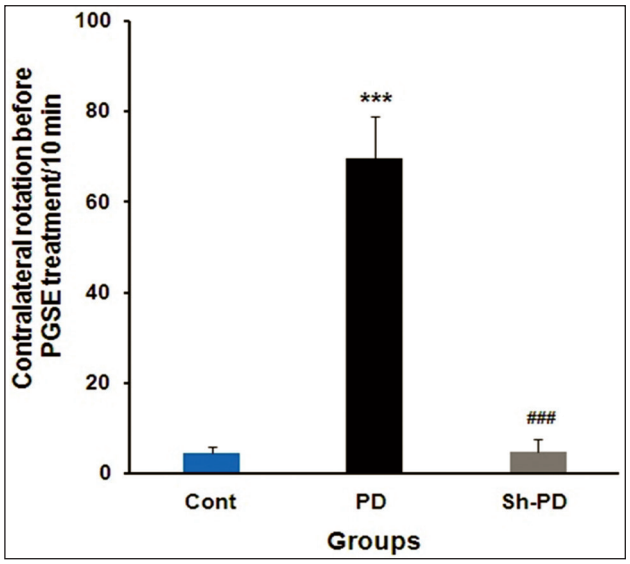

Figure 2. Mean±SEM of contralateral turning numbers after subcutaneous injection of apomorphine to rats with PD as rotation behavior in control, Sh-PD and PD. Rotation number during 10 minutes was higher than control and Sh-PD significantly (P<0.001). One-way ANOVA followed by LSD post hoc test.

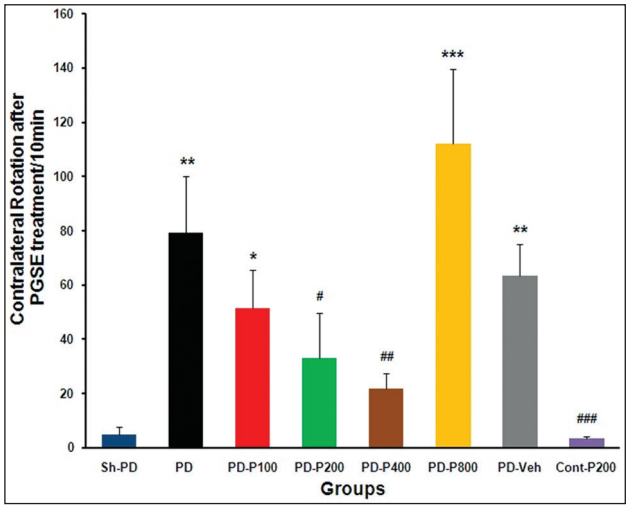

Figure 3. Mean±SEM of contralateral turning numbers after subcutaneous injection of apomorphine to rats with PD as rotation behavior in Sh-PD, PD, rats with suffering PD treated with different doses of PGSE for 14 days after approving the parkinsonian state by apomorphine at 14th day of lesion (PD-P100-800), PD-Veh and Cont-P200 groups. Rotation numbers in PD, PD-Veh, PD-100 and PD-P800 during 10 minutes were higher than control and Sh-PD significantly (P<0.001). Dose 800 mg/kg PGSE had adverse effect on rotation behavior. One-way ANOVA followed by LSD post hoc test.

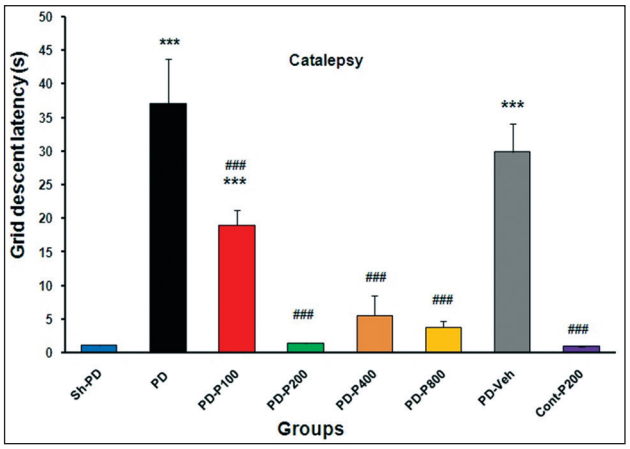

Figure 4. Mean±SEM of grid descent latency (s) as catalepsy in Sh-PD, PD, rats with suffering PD treated with different doses of PGSE for 14 days after approving the parkinsonian state at 14th day of lesion (PD-P100-800), PD-Veh and Cont-P200 groups. Grid descent latency in PD and PD-Veh were higher than Sh-PD and PD rats treated with different doses of PGSE groups significantly (P<0.001). Dose 200 mg/kg PGSE was the best effective dose on catalepsy. Dose 200 mg/kg PGSE didn't affect the catalepsy of intact rats (Cont-P200). One-way ANOVA followed by LSD post hoc test.

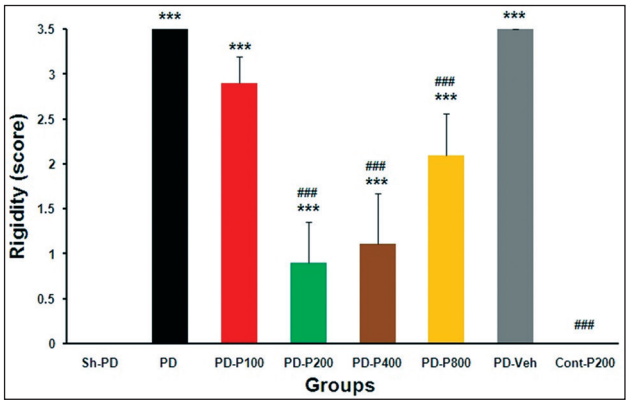

Figure 5. Mean±SEM of rigidity scores as muscle stiffness in Sh-PD, PD, rats with suffering PD treated with different doses of PGSE for 14 days after approving the parkinsonian state at 14th day of lesion (PD-P100-800), PD-Veh and Cont-P200 groups. Rigidity scores in PD, PD-Veh and PD-P100 were significantly higher than Sh-PD and PD rats treated with doses 200-800 mg/kg of PGSE groups (P<0.001). Dose 200 mg/kg PGSE was the best effective dose on rigidity. Dose 200 mg/kg PGSE didn't affect the rigidity of intact rats (Cont-P200). One-way ANOVA followed by LSD post hoc test.

Stride length: Results of forepaws walks length prints (by stride-length test) showed that walk length in PD was significantly lower than that in control or Sh-PD groups (***P<0.001). Treatment of PD rats with PGSE could increase walk length significantly (###P<0.001 for PD-P200-800 vs. PD and PD-Veh, figure 6).

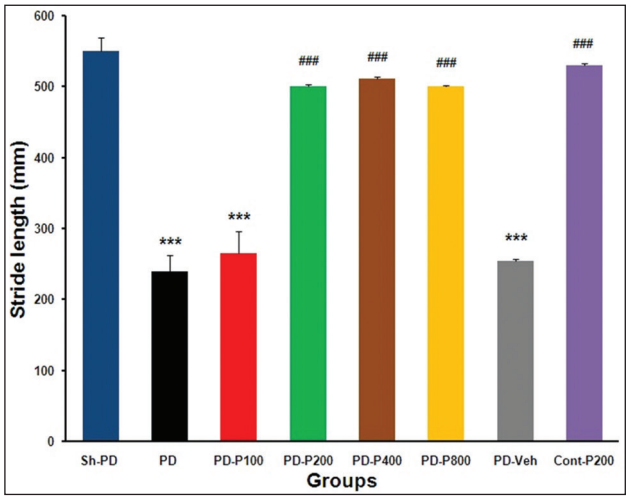

Figure 6. Mean±SEM of stride length (mm) as walks distance in Sh-PD, PD, rats with suffering PD treated with different doses of PGSE for 14 days after approving the parkinsonian state at 14th day of lesion (PD-P100-800), PD-Veh and Cont-P200 groups. Stride length in PD, PD-Veh and PD-P100 were significantly shorter than Sh-PD and PD rats treated with doses 200-800 mg/kg of PGSE groups (P<0.001). Dose 200 mg/kg PGSE was the best effective dose on stride length. Dose 200 mg/kg PGSE didn't affect the stride length of intact rats (Cont-P200). One-way ANOVA followed by LSD post hoc test.

Motor coordination: Data obtained from all groups following motor balance test in rotarod showed that bar descent latency in PD group was decreased severely when compared with control or Sh-PD groups (* P<0.05, **P<0.01 and ***P<0.001for differences between PD, PD-Veh, PD-P100-800 vs. Sh-PD), while treatment with PGSE with doses 200 and 400 mg/kg could improve significantly disrupted motor balance induced by 6-OHDA lesion (###P<0.001 and ##P<0.01for PD-P200 and PD-P400 respectively vs. PD and PD-Veh, figure 7).

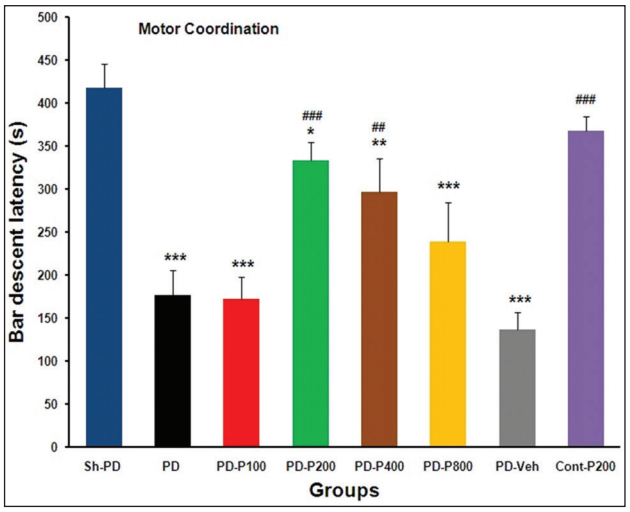

Figure 7. Mean±SEM of bar descent latency (s) as motor coordination on rotarod in Sh-PD, PD, rats with suffering PD treated with different doses of PGSE for 14 days after approving the parkinsonian state at 14th day of lesion (PD-P100-800), PD-Veh and Cont-P200 groups. motor coordination in PD, PD-Veh and PD-P100 were significantly more weak than Sh-PD and PD rats treated with doses 200-800 mg/kg of PGSE groups (P<0.01 and P<0.001). Dose 200 mg/kg PGSE was the best effective dose on motor coordination. Dose 200 mg/kg PGSE didn't affect the coordination of intact rats (Cont-P200). One-way ANOVA followed by LSD post hoc test.

Discussion

The current study presents the novel role of PGSE in the neuroprotection of PD. Specifically, our results demonstrated treatment with PGSE, not only improved the motor performance but also improved avoidance memory deficiency, nociception and inflammation on 6-OHDA induced neurotoxicity. The neuroprotective effects of PGSE were mediated by reducing the production of oxidants and free radicals in brain tissue produced by 6-OHDA. Mounting reports demonstrate that circling frequency of parkinsonian rats is positively correlated with the damage of dopaminergic neurons in the substantia nigra [49]. We demonstrated that PGSE ameliorated the apomorphine-induced circling in rats and attenuated 6-OHDA induced damage of dopaminergic neurons in PD rats thus suggesting its protective effects on dopaminergic neurons. In rotarod, animals walk on

a rotating drum, is widely used to assess motor status in laboratory rodents. Performance is measured by the duration that an animal stays up on the drum as a function of drum speed. This task was provided rich source of information about qualitative aspects of walking movements [50]. It was observed that the mean time taken on rotating drum was less in lesion group and this was attenuated by PGSE treated group, suggesting that the PGSE has neuroprotection in PD on upper and lower extremities as PGSE treated rats have more potential to retain on rota rod. Oxidative stress is a major factor associated with the development and progression of PD [51]. A large number of data suggest that free radicals oxidation damage-particularly of neuronal lipids [52], nucleic acids [53] and proteins [54] are extensive in the brains of PD patients. Increased oxidative stress is thought to result in the generation of free radicals and ROS. Compared to other organs, the brain has been found to be more vulnerable to oxidative stress due to its high lipid content; it's relatively high oxygen metabolism and its low level of antioxidant defenses [55-56]. Markers of oxidative stress, such as lipid peroxidation, GSH, SOD and catalase have been localized to pathologic lesions in the brains of PD patients [57-58]. Inflammation in the brain is a prominent feature of many degenerative diseases of the central nervous system such as PD [59-61]. In addition to the possible involvement in aging, mitochondrial dysfunction and oxidative damage may play important roles in the slowly progressive neuronal death that is characteristic of several different neurodegenerative disorders including PD [61]. The possibility that DA neurons may undergo free radical mediated injury in PD has received support from experiments on animal. There is substantial evidence that the brain which consumes large amounts of oxygen is particularly vulnerable to oxidative damage [61-62].

Glial cells can release deleterious compounds such as proinflammatory cytokines (TNFalpha, Il-1beta), which may act by stimulating nitric oxide production in glial cells, or which may exert a more direct deleterious effect on dopaminergic neurons by activating receptors that contain intracytoplasmic death domains involved in apoptosis [63-64].

It has shown that 6-OHDA neurotoxicity is initiated via extracellular auto-oxidation and the induction of oxidative stress from the oxidative products generated. Neurotoxicity is completely attenuated by preincubation with catalase, suggesting that hydrogen peroxide, at least in part, evokes neuronal cell death in this model. 6-OHDA does not initiate toxicity by dopamine transporter-mediated uptake into PC12 cells. 6-OHDA has previously been shown to induce both apoptotic and necrotic cell-death mechanisms [65].

Pomegranate seed extract (PGSE) has been shown to have anti-inflammatory activity and inhibits lipopolysaccharide-induced inflammatory responses [30].

Pomegranate (Punica granatum) seed linolenic acid isomers were evaluated as selective estrogen receptor modulators (SERMs) in vitro. Punicic acid (PA) inhibited (IC-50) estrogen receptor (ER) alpha at 7.2 microM, ERbeta at 8.8 microM; alpha-eleostearic acid (AEA) inhibited ERalpha/ERbeta at 6.5/7.8 microM [66]. In the same review article, the authors highlighted the major components of pomegranate seeds, juice, pericarp, bark and leaf as well as their pharmacological activity in mammalian cells relevant to the prevention and/or treatment of malignant cell growth, from 2000 to 2006. The mechanisms claimed by the authors referred in that review article included increased apoptosis, decreased inflammation, decreased metastasis and invasion, as well as a decrease in drug resistance [27]. Other review article revealed that pomegranate juice may be fruitful as a therapy for prostate cancer, in atherosclerosis by inhibiting the lipid peroxidation in plasma. Pomegranate juice was also reported effective in hypertension by decreasing Angiotensin-Converting Enzyme (ACE) activity; reducing myocardial ischemia and improving myocardial perfusion; in diabetes through a significant effect on atherogenesis through reduced oxidative stress. Other benefits include the combat to some bacterial infections, erectile dysfunction, male infertility, Alzheimer's disease, obesity. The authors also refer those works concerning the pharmacokinetic of ellagitannins present in pomegranate juice and safety of pomegranate extracts [67]. In another study the toxicology and safety of pomegranate seed oil (PSO) was evaluated by in vitro and in vivo toxicity tests (acute toxicity and 28-day toxicity in Wistar rats). The acute oral toxicity study revealed no significant

findings at 2000 mg PSO/kg body weight. In the 28-day dietary toxicity study PSO was dosed at concentrations in a mean intake of 0-0, 825-847, 4269-4330 and 13,710-14,214 mg PSO/kg body weight per day in males-females, respectively. The no observable adverse effect level (NOAEL) was 4.3 g PSO/kg body weight/day [68]. PG extract similar to imipramine, a recognized antidepressant drug was able to induce a significant decrease in the immobility time. PGSE similar to morphine, a recognized antinociceptive agent exhibited antinociceptive property. Phytochemical investigation of ethanol extract for the presence of phenolic compounds, flavonoids, tannins, anthocyanins, sugars and saponins was also carried out. The CNS activity of ethanol extract of PG seeds may be due to its antioxidative profile [69].

Conclusion

Our findings in this work showed the first evidence that PGSE was effective on inhibiting 6-OHDA neurotoxicity in rats. PGSE protected 6-OHDA induced motor disorders.

Overall our study suggested the potential clinical efficacy of PGSE for improving of Parkinson's disease. We hypothesized that PGSE may as antioxidant could attenuate neuronal oxidative stress, apoptosis and inflammation. It is tempting for the application of PGSE to be useful therapeutic method in patients with Parkinson's disease and other neurodegenerative disorders.

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Evaluation of the effects' hearing aids usage in early period on quality of life in presbyacusis patients

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Abstract

Objectives: Aim of this study is the usage of hearing aids exerts a positive impact on early, anxiety, depression and quality of life in prebyacusis individuals as using the Visual Analog Scale, Hospital Anxiety and Depression Scale and the Short Form-36 scales to evaluate.

Methods: Socio-demographic Data Form, Visual Analog Scale, Hospital Anxiety and Depression Scale and Short-Form-36 Observation Scale were performed. This is a prospective, case-control study. Sixty patients who were diagnosed of presbyacusis with complaints of hearing loss. To analyze the data test significance of the difference between spouses and the Wilcoxon test was used, and error level was set at 0.05.

Results: When the obtained Visual Analog Scale scores were compared with each other, a statistically significant reduction was achieved.

With the comparison of statistical measurements of Hospital Anxiety and Depression Scale of the individuals participating in the study the reduction in anxiety levels were significant.

In the Short-Form 36 Scale of participants after the use of hearing aid statistically significant improvements were determinates in the ways of social function, strengthening of physical role, emotional recruitment, mental health, general perception of energy/vitality and health.

Conculusion: Patients with age-related hearing loss should be followed in terms of psychosocial view after hearing aid, and it should not be ignored that patient must be dealt in terms of all these factors as a whole.

Key words: Presbycusis, life-quality, hearing aid, visual analogue scale, hospital anxiety and depression scale, short-form 36.

Introduction

Functional decline of the nervous system is a cardinal feature of normal aging [1, 2]. Age related hearing loss (presbycusis-ARHL) is the third most prevalent condition of elderly persons, exceeded only by arthritis and hypertension, with approximately 97% of people experiencing a decline in hearing during aging [3, 4]. Presbycusis is defined as sensorineural hearing loss, which varies between mild to profound in the low as well as the high frequencies, having a gradual and progressive onset, symmetrical, descending and bilateral for high frequency sounds (3 to 8KHz), often times followed by difficulties in speech recognition [5-9]. Presbycusis is also characterized by reduced speech understanding in noisy environments, slowed central processing of acoustic information, and impaired sound localization.

Hearing loss is one of the sensorial deficits which has the most impact on the lives of people; because it impairs the person's capacity to effectively engage in communication. Thus, there is a reduction on speech intelligibility, impairing verbal communication, interfering in receiving information, forming and expressing one's ideas. Moreover, there is patient isolation, reduced socialization and intolerance to moderate to high-intensity sounds [10-12].

Because of that, in our study, it is intended to evaluate the positive effects of hearing aids usage can display on in early (first 1 month), anxiety, depression and quality of life in individuals with ARHL as using the Visual Analog Scale (VAS), Hospital Anxiety and Depression Scale (HAD) and Short Form 36 (SF36) scales.

Material and methods

Sixty patients who consult with hearing loss to Cumhuriyet University Faculty of Medicine, Ear, Nose and Throat major field from January to May of 2011, and also who diagnosed presbyacusis and agreed to participate, were included in the study.

For the research, it was taken an approval from Cumhuriyet University Clinical Research Ethics Committee and a signed informed consent paper from all patients.

It was accepted as criteria that was excluded exterior in our research who being the prevention level of physical illness or cognitive insufficiency to make conversation or fill out Scales, and also who are still being treated for a psychiatric illness, and to deny making approval to participate in the study.

All of the cases participating in the study a complete ear, nose and throat and head and neck examination and pure tone audiometric examination were performed. As a result of Pure-tone audiometric examination diagnosed as ARHL and recommended to use hearing aids 60 [33 (55%) were male and 27 (45%) females] , it was applied in the following to all of the patient's before started to use hearing aids and after 1 month of the hearing aids a demographic data form, VAS, HAD and SF-36 scale.

In the form of socio-demographic data which was prepared by researchers, it was questioned patient age, gender, educational status, marital status, smoking and alcohol addiction and a history of chronic disease.

The VAS scale was applied to evaluate personal perceptions on changes in hearing all cases participating in the study. According to this, it was asked to give a score between 0 and 10 to their hearing levels. It was accepted as 0 point as hearing level is normal and 10 point as total level of hearing loss.

Hospital Anxiety and Depression Scale (HADS) developed by Zigmond and Snaith [13] were administered to all subjects in the preoperative period in order to determine the levels of anxiety and depression and to measure the change in their severity. This scale was adapted to Turkish and reliability and validity findings were published by Aydemir et al [14]. The HADS is a 14-item scale with two subscales; one measuring depression (HADS-D), the other measuring anxiety (HADS-A). Seven of them (odd numbers), are measuring anxiety, and the other seven (even numbers), are measuring depression, psychotic disorders. Each item is rated from 0 to 3. Scores are summed up separately. As a result of the work for Turkey, it is found that the anxiety subscale cut-off score 10/11, and the depression subscale of the 7/8. According to this, the cases with ARHL who takes on these points evaluated as risk group. Due to lack of physical symptoms, related to the HAD scale, it was chosen in this study.

In order to evaluate SF-36 individuals' the quality of life, it was developed by Rand Corporation [15].Translation into Turkish, validity and reliability study was made by the Kocyigit et al. [16] in 1999. SF-36 physical function consist of 36 questions, social function, role limitations due to physical problems, role limitations due to emotional problems, mental health, energy/vitality, pain and general health perception. The subscales evaluate the health between 0-100 and 0 poor health status, 100 indicates the status of good health.

The SPSS 14.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. To analyze the data test significance of the difference between spouses and the Wilcoxon test was used, and error level was set at 0.05.

Results

It was identified that ages of patients who participated in the study ranged from 41 to 84, the average age was 63.02 ± 12.08 years. It was identified as 33 male patients (55%), 27 female (45%) and the mean age for male 62.67 ± 11.06 years, female 63.44 ± 13.43 years, respectively.

All of patients in the study were evaluated in terms of their evaluation form of socio-demographic, status of the education, employment status, marital status, chronic illness, smoking and alcohol usage (Table 1).

Before (VAS 1), and a month after (VAS 2) using a hearing aid to evaluate the changes on personal perceptions the scoring was made in VAS between 0-10. While the value of VAS 1's average cases 4.95 ± 1.77, VAS 2 value was 3.06 ± 1.71. Compared with the VAS scores obtained after the use of a hearing aid were found statistically significant reduction ($p < 0.05$). According to gender average values of VAS scores are shown in Table 2.

For evaluating the changes anxiety and depression levels in patients who participate in this study compared before using a hearing aids and applied HAD scale's data, after using a month; while after using hearing aids were found unimportant in the level of depression ($p > 0.05$), a significant reduction was observed in the level of anxiety ($t = 2.31$ $p = 0.024$).

Table 1. Distribution of socio-demographic data according to gender age related hearing loss of the 60 patients

		Women	Men
Educational level	Illiterate	13 (%48,1)	5 (%15,2)
	Literate	4 (14,8)	3 (%9,1)
	Primary school	8 (29,6)	18 (%54,5)
	High school	2 (7,4)	7 (%21,2)
Occupation	Employed	1 (%3,7)	5 (%15,2)
	Unemployed	26 (%96,3)	28 (%84,8)
Marital status	Married	20 (%74,1)	30 (%90,9)
	Single	1 (%3,7)	0 (%0)
	His wife died	6 (%22,2)	2 (%6,1)
	Divorced	0 (%0)	1 (%3,0)
Choric disease	Yes	18 (%66,7)	18 (%54,5)
	No	9 (%33,3)	5 (%45,5)
Cigarette use	Active smoker	3 (%11,1)	16 (%48,5)
	Nonsmoker	24 (%88,9)	17 (%51,5)
Alcohol use	Yes	0 (%0)	4 (%12,1)
	No	27 (%100)	29 (%87,9)

Table 2. According to the gender distribution of the change in VAS scores (means ±SDs)

Gender	VAS	Means	Result
Women	VAS1	4,5926±1,62	P=0.001*
	VAS2	3,0370±1,74	
Men	VAS1	5,2424±1,85	P=0.001*

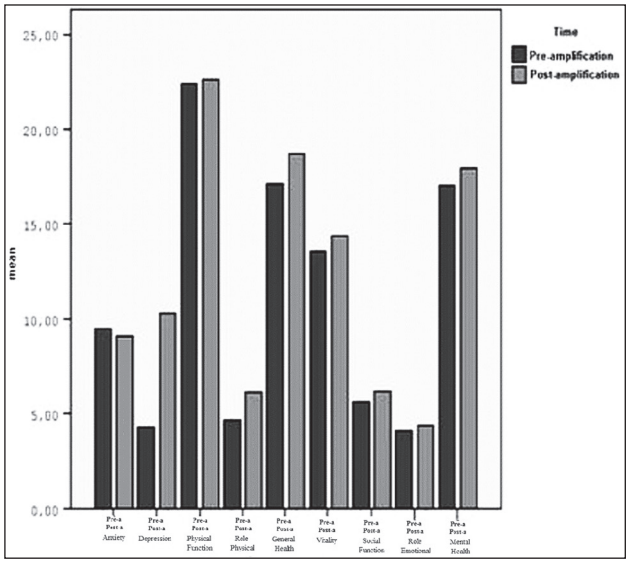
Table 3. All individuals included in the study, before and one month after starting to use a hearing aid results' distribution which was obtained HAD and SF-36 assessment scales (means ±SDs)

		Mean	Result
Anxiety	Before using a hearing aids	8,8333±4,05	t = 2.31 p=0.024*
	After using a hearing aids	7,9500±4,95	
Depression	Before using a hearing aids	9,4333±3,82	t=1.93 p=0.058
	After using a hearing aids	8,6000±4,79	
Physical function	Before using a hearing aids	23,9833±5,89	t=1.10 p=0.274
	After using a hearing aids	24,2333±5,58	
Physical role in the reinforcement	Before using a hearing aids	4,8000±1,41	t=8.03 p=0.001*
	After using a hearing aids	6,6167±1,70	
General health perception	Before using a hearing aids	17,1500±3,01	t=3.79 p=0.001*
	After using a hearing aids	18,3500 ±2,91	
Vitality	Before using a hearing aids	14,8833±4,34	t=2.26 p=0.027*
	After using a hearing aids	15,4167±4,27	
Social functioning	Before using a hearing aids	6,1667±2,10	t=2.89 p=0.005*
	After using a hearing aids	6,8333±2,59	
Strengthen the role emotional	Before using a hearing aids	4,6500±1,42	t=2.22 p=0.030*
	After using a hearing aids	4,9500±1,56	
Mental health	Before using a hearing aids	18,3833±4,88	t=3.34 p=0.001*
	After using a hearing aids	19,4833±5,04	

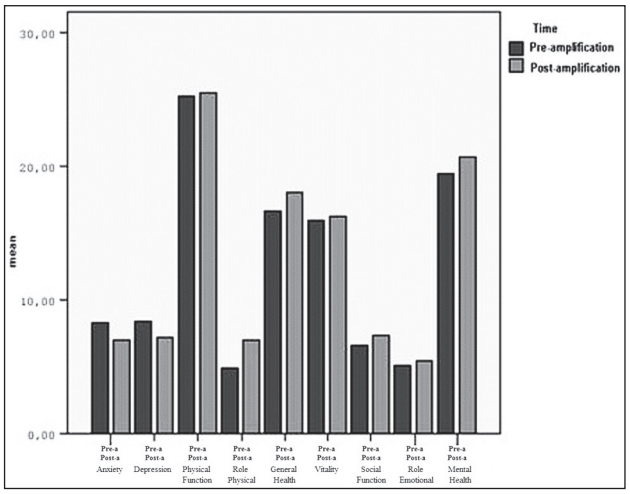
According to the data obtained from the SF-36 scale of all cases, while after the use of a hearing aids was found unimportant in terms of physical function (p>0.05), social functioning (t=2.89, p=0.005), physical role in the reinforcement (t=8.03, p=0.001), strengthen the role emotional (t=2.22, p=0.030), mental health (t=3.34, p=0.001), energy/vitality (t=2.22, p=0.027) and general health perception (t=3.79, p=0.001) was statistically significant showed an improvement (Table 3).

When evaluated the patients who participate in the study separated according to gender, their data which was obtained HAD and SF-36 scale; female individuals after using a hearing aids a significant increase to strengthen the role in scores was found vitality (t=2.20, p=0.037), emotional (t=2.05, p=0.05) and physical (t=4.54, p=0.001) and the male cases reduction in scores anxiety (t=2.94, p=0.006) and depression (t=2.21, p=0.034) ;and a significant increase in that scores showed; physical role reinforcement (t=6.77, p=0.001), general health (t=3.23, p=0.003), social functioning (t=2.62, p=0.013) and mental health (t=2.78, p=0.009).

All individuals included in the study before and one month after starting to use a hearing aids the results' distribution according to sex which was obtained HAD and SF-36 assessment scales are shown in Graph 1 and Graph 2.



Graphic 1. Distribution of anxiety, depression and paremeters of quality of life in female



Graphic 2. Distribution of anxiety, depression and paremeters of quality of life in male

Discussion

The hearing apparatus is one of the most important factors related to the development of oral communication. Hearing impairment is a multi faceted condition with medical and social aspects.

ARHL is a complex disease with multi factorial etiology. It is the most prevalent sensory impairment in the elderly, and may have detrimental effects on their quality of life and psychological well-being. Left untreated, presbycusis can not only lead sufferers to reduced quality of life, isolation, dependence and frustration, but also affect the healthy people around. For this reason nowadays, there is a noticeable world tendency towards improving hard of hearing person's quality of life. The effect of using a hearing aid in changing the quality of life of the hearing-impaired elderly people has been investigated in many studies.

Tesch-Römer [17] examined elderly individuals with mild to moderate hearing loss who received a hearing aid for the first time in their lives whose performance in the domains of communication problems, social activities, satisfaction with social relationships, well-being, and cognition. Data analyses show that in older persons with mild to moderate hearing loss, hearing aid use has positive effects on self-perceived hearing handicap, but there is no effect of hearing aid use in domains like social activities, satisfaction with social relations, well-being, and cognitive functioning [17].

The effect of an appropriate hearing aid on communicative efficiency was investigated by

Harless et al. [18] and their finding suggested that improvement in communicative efficiency, achieved through use of appropriate wearable amplification, may bear some relation to the self image of older hearing-impaired individuals.

Mulrow et al [19] were investigated the effect of an appropriate hearing aid on quality of life and they found hearing loss is associated with important adverse effects on the quality of life of elderly persons, effects which are reversible with hearing aids.

Lotfi et al. [20] were to investigate the quality of life in elderly people who are hard of hearing after wearing a hearing aid. Hearing Handicap Inventory for the Elderly (HHIE) questionnaire before and three months after using a hearing aid and 207 patients evaluated in this study. And their results showed a significant improvement of the quality of life after three months of using a hearing aid in all participants and betterment of their most important problems i.e., the communication and exchange of information.

Chen et al.[21] investigate the effect on quality of life to usage of hearing aids in patients ARHL in this study which HHIE questionnaire and the SADL (satisfaction with amplification in daily life) questionnaire is used, these surveys, which show the effects of usage hearing aids in early period, utilizes a reliable scales to assess the effects .

The aim of Acar et al.'s [22] study was to report the cognitive and psychological benefits of using hearing aids by the elderly people, over the age of 65. Thirty-four elderly subjects with hearing impairment who answered the geriatric depression scale-short form (GDS) questionnaire and the mini mental state examination (MMSE) test, prior to, and 3 months following the use of hearing aid. All patients showed a significant improvement of the psychosocial and cognitive conditions. Dalton et al [23] investigate the impact of hearing loss on quality of life in 2,688 older adults. Difficulties with communication were assessed by using the Hearing Handicap for the Elderly-Screening version (HHIE-S), health-related quality of life was assessed by using measures of activities of daily living (ADLs), instrumental ADLs (IADLs) and the Short Form 36 Health Survey (SF-36). Severity of hearing loss was significantly associated with decreased function in both the Mental Component Summary score and the Physical Component Summary score of the SF-36 as well as with six of the eight individual domain scores. In this study, after using a hearing aids while there were found no differences in SF-36 scores in terms of physical function, it showed a significant improvement in social function, physical role in the strengthening, strengthening the emotional role, mental health, energy/vitality and health's general in the perception.

Hogan et al [24] examines the health effects associated with self-reported hearing disability on older people. In this study the SF-12 scale has been used for assessing the quality of life for patients. The SF-12 provides summary measures for physical and mental health and has been shown to be a practical alternative to the SF-36 for the purposes of large group comparisons on overall physical and mental health outcomes [25]. Their results compared with population norms, hearing disability at all levels was associated with poorer physical and mental health scores on the SF-12 measure, especially for people with severe or profound hearing loss, thus suggesting a threshold effect at advanced levels of disability.

Vuorialho et al. [26] were evaluated 98 individuals with ARHL effect of usage a hearing aids on quality of life as using HHIE-S, EuroQol questionnaire (EQ, EuroQol Questionnaire) and the VAS scoring. While in this study, 6 months after using hearing aids, scores of individuals' EQ and VAS was detected in a significant improvement, according to HHIE-S scores show to be less in social and emotional problems than that. In addition the study of Vuorialho et al [26] in patients ARHL the EQ-5D questionnaire study was not sensitive enough for measuring the health-related quality of life of subjects with hearing impairment shown to be. Sixty individuals participate in this study, which use a hearing aid for evaluation the change hearing loss in early stage that personal perceptions has been applied VAS, all patients VAS score's a significant improvement was shown in VAS scores.

Stark and Hickson [27] examined the effect that hearing impairment and aural rehabilitation has on the person with hearing impairment and the significant others (SO) quality of life (QOL). In this study, the evaluation of quality of life before and three months after starting to use a hearing aid HHIE, the SF-36 scale has been used in Denver and quantified the results obtained emphasize the

significant impact of hearing impairment on both the person with hearing impairment and the SO.

There were a lot of studies in the literature on the psychological problems caused by hearing loss in individuals [17, 28- 31]. Studies have evaluated the quality of life of individuals ARHL often HHIE [20, 21], HHIE-S [22, 32], GDS [21], MMSE [22], SADL [21], hearing-specific QOL, health-related QOL , SF-36 and significant others (SO) completed a modified version of the quantified Denver Scale [27], the SF-36 [23], VAS [26] is located on more than we used in our literature studies ARHL research of individuals' VAS quality of life, we didn't come across using the SF36 and HAD scales the association together. In this study, comparing before and after the physical, mental, and social change felt patients who use a hearing aids the SF36 scale, in the evaluation of the effects of anxiety and depression HAD scale has been used. While hearing aid usage in individuals with ARHL a significant impact on the level of depression isn't shown, in anxiety levels causing statistically a decrease have been found. But we think that we can't report a definitive judicial about this subject because depression and anxiety are a chronic diseases which can treat a long time and sometimes individuals can demonstrate resistance to treatment . For this reason treatment which longs as little as one month, as well as changes occurred, we think that it is more accurate to plan evaluated studies which the changing occurs in long term in the future. Because the use of hearing aids is extremely important as how it affects individuals with ARHL after short-term changes as how change the satisfaction.

The most important limitation of this study is the use of hearing aids with primary ARHL patients on quality of life investigated, but the relationship between the effects of hearing loss to occur the frequency range, and the quality of life has not been evaluated. The usage of hearing aids with ARHL cases, participated in literature the studies investigating the effects on quality of life in many of these points are not discussed. We are on the opinion that in future studies on this subject, it will be useful to investigate whether there is a relation between individuals hearing loss seen in the frequency range and the improvement in quality of life.

Conclusion

The usage of hearing aids in ARHL cases evaluation studies in the literature investigating the effects on quality of life is usually made after the first three months of usage. This study is the first which HAD scale is used and which evaluated the usage of hearing aids ARHL cases, in most early period the effects on quality of life. As a result, in this study quality of life for patients ARHL after the usage of hearing aids in the early period also been shown to be many changes in a positive way. These changes aren't only related to the recovery of hearing individuals but also the psychological, social and family lives. For this reason, especially after hearing aids, ARHL patients not only in terms of gains hearing but also in the psychosocial aspects and patients is be followed in terms of all these factors must be considered as a whole should not be ignored.

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Drug dependency and substance abuse following prescription of opioid analgesics among cancer patients in Mazandaran province of Iran

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Abstract

Background: Opioid analgesics are frequently used in cancer patients. Drug dependency and substance abuse are matter of concerns in patients taking these analgesics.

Objective: Evaluating the prevalence of substance abuse and drug dependency among cancer patients as a result of treatment with opioids in order to deplete pain.

Study design: descriptive, cross-sectional, retrospective study

Setting: Food and Drug council of Mazandaran University of Medical Sciences

Methods: Patients, who were referred for receiving opioid analgesic prescription, were carefully interviewed to record the frequency of the substance abuse and drug dependency through a Structured Clinical Interview for Axis I disorder with and SCID I according DSM IV.

Results: Among 238 cancer patients, 27 (11.3%) patients were drug dependent and 24 (9.9%) patients had a substance abuse. Most of the patients, who were either dependent or abuser, were married and also had no schoolfellow education. Methadone was the major prescribed opioid analgesic that depended patients, and the most substance abused out of the prescription was opium.

Conclusion: High prevalence of the drug dependency and substance abuse among cancer patients in Iran requires considerable attention and further studies to find out the underlying causes. Furthermore, the prescribed analgesics dosages were not adequate enough to relief the pain of cancer patients so that patients have to take other substances with stronger analgesic effects that it could cause harmful consequences.

Key words: Opioid analgesics, cancer pain, abuse, dependency.

Introduction

Cancer pain is observed in 40-50% of patients with metastatic cancer and 90% of patients with advanced cancer (1). In the recent years, depletion of the pain among these patients has been one of the main goals of treatment (2) and is known as a "patient's right" (3-4).

In mild to moderate pains, NSAIDs or antidepressants are being used as monotherapy or in combination with Antiepileptic (7-8). As their efficacy is not comparable to an opioid analgesic, opioids have recently been the major analgesic drugs prescribed by the physician in cancer patients.

Usage of opioids has several limitations. Different factors such existing policies, ongoing ignorance by medical staff and related side effects may prevent the prescription of an appropriate drug (9-10). Drug tolerance is one of the most important side effects with significant harmful consequences (12). Patients' situation and the severity of the pain are two other important points to choose an appropriate drug because drugs have different onset and duration of actions (11).

Drug dependency and substance abuse are the main concern in the prescription of opioid analgesics among cancer patients because of their either ability to cause dependency or abusing property (15). Improper pain management or prescription of the higher doses of opioid drugs in cancer patients would lead to some addiction-like behaviors and may cause the symptoms of dependency. This is also mentioned as iatrogenic addiction or pseudo addiction (16).

Many reports showed that patients usually raise the initial dosage of opioids are to make the initial efficacy (13-14). In spite of these concerns, there are no reliable data regarding neither the frequency of the improper pain killers consumption,

nor the prevalence of drug dependency or substance abuses among these patients.

To our best knowledge, till now, no study with the aim of evaluating the frequency of improper pain killer consumption and the prevalence of drug dependency and substance abuse have been published previously in any international medical journals. So, the aima of this study was to evaluate the prevalence of opioid analgesics dependency and substance abuse among cancer patients

Patients and methods

This descriptive, cross-sectional, retrospective study was conducted at the Food and Drug council of Mazandaran University of Medical Sciences in North of Iran during 2008 to2009. The study was approved by the research committee of Mazandaran University of Medical Sciences. In Mazandaran province, many patients who need to receive opioid analgesics prescription are referred to this unit. Cases were selected among cancer patients who suffered from severe pain and had an approvement to receive opioid analgesics for more than six months according to their clinical features and histopathological results proposed in the medical committee.

Patients, who their histopathological results were not approved by medical committee or their duration of treatment was less than six months were excluded.

A total of 328 patients with drug addiction were interviewed to evaluation of the substance abuse and dependency was base on an structured Clinical Interview for Axis I disorder with SCID I according to the DSM IV criteria, which their validity & reliability were tested previously in normal Iranian population (17). In addition, another study determined three other characteristics for substance abuse and dependency which were overdose, compulsive use and continued use despite damage (18).

In the current study, interviews were performed by general practitioners whom were already trained in this area. The interviews were performed when patients were referred to receive the opioid analgesics medications.

The dosages of analgesics were different depend on the patients' pain severity and cancer types. Drug dependency was considered if the patients used opioid analgesics more than the prescribed dosages. Substance abuse was defined as demonstrating additional signs and/or symptoms of dependency in patients who took the opioids out of the prescription.

The demographic information including gender, age, marital status, educational level, type of opioid analgesic, history of substance abuse, symptoms of dependency, type of the cancer were collected from the patient's case note made by interviewing from the patients.

According to the Helsinki agreement, the study goals and the patients' rights were initially described for all patients and an informed written consent was obtained. The questionnaire and written consent will be provided upon any request.

Statistical Package for Social Sciences (SPSS), Version 14.0. , was used for the statistical analysis. Continuous variables were compared using student's t test or one-way analysis of variance (ANOVA) as appropriate among the groups. P-value of less than 0.05 was considered as significant.

Results

During the period of this study, 912 cancer patients were referred to the Food and Drug Council of Mazandaran University of Medical Sciences and among them 328 patients had criteria to enter the the study. in which 88 (37%) cases were females and 150 (63%) males. The mean age of the patients was 57±13.7 and 62±15.3 years for females and males, respectively.(Table 1)

Table 1. Marital status in cancer patients with the drug dependency or substance abuse

Marital status	Drug dependency		Substance abuse	
	Positive	Negative	Positive	Negative
	n (%)	n (%)	n (%)	n (%)
Single	1 (3.7%)	3 (1.4%)	0 (0.0%)	4 (1.9%)
Married	26 (96.3%)	208 (98.6%)	24 (100%)	210 (98.1%)
Total	27 (11.3%)	211 (88.7%)	24 (10%)	214 (90%)

Among all, 234 (98.3%) patients were married and only 4 (1.7%) were single. 27 (11.3%) patients (18 males and 9 females) met the criteria of the drug dependency (group A). (Table 1)

The mean age in this group was 52.9±19.3 and the mean duration of cancer among them was 5±4.7 years. (Table 1)

24 (10%) patients (19 males and 5 females) met the criteria of the substance abuse definition (group B). The mean age in this group was 64.1±14.2 and the mean duration of cancer among them was 7.9±4.2 years. 26 patients in group A and all of the patients in group B were married. (Table 1) Most of the patients in both groups had no schoolfellow education or had an educational level lower than the guidance school (Table 1, 2).

Prevalence of drug dependency and substance abuse in different cancer types is shown in Table 3. The most common drug dependency and substance abused were seen among patients with bone involvement (primary or metastatic) (26.9%) and gastric cancer (20.9%), respectively. (Table 3)

Average duration of treatment by analgesics in group A (What is it's definition?) was 8.1 ± 4.2 years. Most of the patients were treated by methadone tablets and a few percent of the patients were treated by other opioid analgesic drugs (Table 4).

Table 4. Frequency of Opioid analgesics prescribed in cancer patients in Mazandaran

Analgesic Type	n	%
Methadone Tab	199	83.60%
Methadone Amp	10	4.20%
Morphine Amp	27	11.30%
Pethidine Amp	1	0.40%
Pentazocibe Amp	1	0.40%
Total	238	100%

Although 140 (47.9%) patients in this study had no previous history of substance abuses, 124 (52.1%) patients had the history of a substance abuse before the prescription of opioids analgesics. Among patients who had the history of the substance abuse, opium was the most common substance taken by 74 patients. None of the patients mentioned the history of alcohol abuse.

Most of the patients in group A (85.1%) were depended to methadone (Table 5). Among 24 patients in group B, 14 (58.3%) of them abused the opium out of the prescription (Table 6). The mean duration of drug dependency among patients in group A and substance abuse in group B was 4.5 ± 2.8 and 5.2 ± 2.9 years, respectively.

There was statistically significant drug dependency prevalence difference between the ages of

Table 2. Educational level in cancer patients with the drug addiction or substance abuse

Educational Level	Drug Dependency		Substance Abuse		Total
	Positive	Negative	Positive	Negative	
	n (%)	n (%)	n(%)	n (%)	
No Education	12 (44.4%)	47 (22.3%)	8 (33.3%)	51 (23.8%)	59
Primary School	3 (11.1%)	27 (12.8%)	3 (12.5%)	27 (12.6%)	30
Guidance school	9 (33.3%)	86 (40.6%)	8 (33.3%)	87 (40.7%)	95
High school or higher	3 (11.2%)	51 (24.2%)	5 (20.8%)	49 (22.9%)	54
Total	27 (100%)	211 (100%)	24 (100%)	214 (100%)	238

Table 3. The relation between the drug dependency and substance abuse prevalence with cancer types

Cancer Type	Drug Dependency		Substance abuse		Total*
	Positive	Negative	Positive	Negative	
	n (%)	n (%)	n (%)	n (%)	
Colorectal	3 (13%)	20 (87%)	1 (4.3%)	22 (95.7%)	23
Breast	2 (6.7%)	28 (93.3%)	2 (6.7%)	28 (93.3%)	30
Gastric	3 (7%)	40 (93%)	9 (20.9%)	34 (79.1%)	43
Prostate	3(12%)	22 (88%)	3 (12%)	22 (88%)	25
Primary or metastatic Bone	7 (26.9%)	73 (73.1%)	5 (19.2%)	21 (80.8%)	26

** Some cases excluded from the study so that the total number of this table is less than the total patients*

patients that addiction-like behaviors was more common in older patients.

Table 5. Drugs among depended cancer patients with more dependency symptoms

Drug abuse	N	%
Methadone tab	23	85.10%
Morphine amp	4	14.90%

Table 6. Substances among abused patients taken out of prescription

Substance	N	%
Opium	14	58.30%
Codeine Tab	4	16.70%
Tramadole	2	8.30%
Bupronorphine	1	4.20%
Benzodiazepines	3	12.50%

Also, there was contrarily statistical significant between duration of opioid analgesic intakes and drug dependency. The longer duration of medications caused the less dependency behaviors.

Also, the relation between drug dependency and substance abuse was statistically significant; the abuse increased following by increasing of dependency behaviors.

There was no statistically significant difference between medication's dosage and dependency. A Significant difference was seen between duration of the cancer and drug dependency. Most of substance abusers had longer duration of the cancer.

There was no statistically significant difference between substance abuse with the age of Patients or medications' dosage . Also, there was not any significant difference between drug dependency and substance abuse with the type of the cancers.

Discussion

In the current study, the prevalence of drug dependency and substance abuse among cancer patients were studied as a consequence of consumption of opioid analgesics in order to relief the pain. The usage of these kinds of drugs is forbidden among healthy people in the country, because of their ability to cause dependency and addiction. These drugs have also recently being abused, particularly among young population, so that the stick rules have been passed to limit the consumption of them in the society.

Substance abuse or drug dependency in older patients was higher when it compared to the younger patients. This result may be related to this point that older patients had lower pain threshold so they should take the medicine with more potency.

Patients, who were treated for a long time period, though were less lilely to have drug dependency; they had more tendencies to abuse the substance. One of the reasons could be related to the lack of their self-confidence after a long time treatment. They disappointed from the treatment so that they turn their faces into another stronger substance in order to heal their pain. In contrast, the newer patients were overdosed in the treatment procedure. They increased their medications doses to get rid of the pain as prompt as possible.

In this study, drug abuse and substance dependency were significantly higher in married patients. This could be justified from this point that the married patients prefer to have a better life style with their family without suffering from the pain.

Many studies showed that patients, who had less educational level, were more vulnerable to pain (9-21).

We similarly observed that most of the patients, who were suffer from substance abuse or drug dependency, were illiterated and had less educational levels.

Although different studies associated with cancer pain have shown that the special kinds of cancers such as bone, pancreas and esophagus cancers have more pain levels (22-23), several studies showed that the incidence of pain and its severity had no relation with cancer types(24-25).

In the current study, substance abuse and drug dependency were most common among bone (primary or metastatic) and gastric cancer patients, respectively. Unfortunately, some patients had left the study before it finished. Therefore their data was not complete and excluded from the study.

Morphine is the most used opioid analgesic drug for pain management in the world (26). Over the past few years, morphine has been proved as a potent agent to suppress the clinical pains. In several studies, morphine has been compared to oxycodone (27), hydromorphone (28), phentanyle (29) and methadone (30), and it has been trying to find a replacement for it, but no substitutive results were achieved. Nevertheless, due to the low num-

ber of patients included in these studies, reliability is questioned. In the present study, 87.8% of patients were taken methadone tablets or injections, and morphine were used only in 11%. Due to the lack of enough knowledge to prescribe appropriate doses of methadone to each patient, it might be concluded that this drug is not a good choice in the pain management of the cancer patients. Prescribing an appropriate dosage of methadone would make it an acceptable analgesic with high efficacy, tolerability and convenience.

Although there is no similar study in the prevalence of substance abuse and drug dependency among cancer patients in Iran, several studies has been conducted in other countries. Jette Hojsted (31) performed a systematic review in 2006. The prevalence of substance abuse and dependency in his study was 9.1% and 37% , respectively, according to DSM -IV criteria.

Also, some studies showed that the prevalence of drug abuse in non-cancer patients was 24% (32).

Heroin is the most abused substance in European or American countries (33). However, there is no accurate information about the consumption of it. In the present study, a large number of patients were relatively dependent to opium. The reasons may be availability of opium in Iran and its easy injection compared to other injection opioids.

Some previous studies were evaluated the improper physicians' knowledge about the pain management of cancer patients (34, 35). In addition, many studies showed that pains could be suppressible among 70-90% of cancer patients if a proper analgesic dose or a proper guideline is used (36-38). In the recent years, this rate has changed into 24-60% in patients who were actively treated with anti-cancer drugs (39-41) and 62% -86% in patients with advanced cancer (2-44).

Current retrospective study had some limitations that might affect the final results. The most fundamental problem with the quality of all retrospective studies is the lack of accurate information and missing data. Many patients may be reluctant to disclose any past history of substance abuse because of concerning about negative attitudes or undue suspicion that would lead to underestimate the prevalence. In spite of this fact, the results of the current study contain valuable information and may be worth further investigation and elucidation.

Conclusion

This study has shown that the prevalence of drug dependency and substance abuse are high among cancer patients in Iran require considerable attention. Patients should be continuously monitored during the pain management with opioid analgesics. Aproperiate administration of opioids, proper drug forms (intravenous or oral), adequate dosages, regular monitoring, observation of side effects and drug interactions, and patients drug history should be attended otherwise prescription of opioid analgesics can lead to drug abuse or substance dependency.

On the other hand, preparing standard treatment guidelines approved by the University treatment committee, cooperation with Ministry of Health and Medical Education and improving the level of the services to cancer patients can prevent complicated problems. Establishing specific "pain management database for cancer patients" and Designing an study to evaluate the level of pain control, substance abuse and dependency in all cancer patients and also interventional studies to improve their pain score is recommended.

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The susceptibility of orthodontic aesthetic brackets to staining - An in vitro study

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Abstract

Introduction: The high esthetic value of orthodontic appliance elements during treatment becomes an important factor for increasing number of adult patients.

Objectives: The aim of the study was an assessment if food dyes could significantly affect the color of orthodontic brackets.

Material and methods: Eight brands of orthodontic brackets; including polymer-based ones: Rave (Ortho Technology), Crystal Clear Plastic Bracket (Dentsply Glenroe), Silkon Plus (American Orthodontics), Orthoflex (Ortho Technology), and ceramic ones: Reflections (OrthoTechnology), Pure (Ortho-Technology), Contour (Class One Orthodontic Products), Miso (HT Corp.), were investigated in the present study. Ten samples of each kind were prepared. Their color was assessed with the use of Spectroshade (MHT) dental spectrophotometer. Then five brackets of each series were stored in coffee and five in red wine for 24 hours. The color change after storage was analyzed for each sample according to L*a*b* color scale. The lightness, redness, yellowness and complete color change of examined brackets were analyzed statistically at the level of p=0.05.

Results: The highest mean color change ΔE was observed for Silkon brackets after red wine storage at the level of 24.52 points whereas the lowest change was obtained for Contour brand after coffee storage at the level of 1.82 points. The correlations between bracket brand, kind of food colorant and intensity of color change were also noted.

Conclusions: The orthodontic ceramic brackets seem to be susceptible to discoloration by food dyes from external environment. The color change of examined brackets is both material (brand) and food dye dependent.

Key words: Orthodontic brackets, ceramics, composite, discoloration, colorimetric assessment.

Introduction

Fixed orthodontic appliances are a commonly used tool during malocclusion treatment in children and adults. Traditional fixed appliances applied in edgewise and straight wire techniques consist of rings or tubes set on molars and of brackets fastened to labial surfaces of premolars, incisors and canines. Orthodontic brackets can be made of stainless steel, gold alloys, composite materials, polycarbonates or ceramics. The kind of material used determines their physical properties, such as resistance to mechanical force, brittleness, resistance to deformation and optical properties, such as color and transparency.

Because orthodontic brackets are the most visible elements of appliances due to their size and location, their appearance is of high significance, especially from the patient's point of view, who expects not only a satisfactory end effect of treatment i.e. arrangement of teeth and harmonious face features, but also aesthetic appearance of dental arches during a two-year-long, on average, use of fixed appliances.

There are two types of clinical procedures resulting in reduced visibility of dental appliances in the patient's oral cavity. The first procedure consists in placing elements of braces on lingual and palatal surfaces of teeth (lingual techniques), the other one uses elements - fixed to labial surfaces of teeth - whose color is similar to that of dentition (traditional treatment techniques).

Although fixed appliances stuck onto lingual and palatal surfaces of teeth fulfill aesthetic criteria to a high degree, the techniques have certain limitations resulting from their lower efficacy, harder conditions for dentist's work or extended time and increased costs of treatment[1]. Therefore a greater popularity lies with methods using fixed appliances stuck onto labial surfaces of teeth.

Producers of orthodontic materials, in order to meet their clients' expectations, are trying to replace steel alloys with materials whose color resembles that of patient's dentition. Aesthetic brackets, similar in color to enamel and dentine can be made from polycarbonates, composite materials or ceramics based on aluminum oxide Al2O3.

Polycarbonate plastics and polymers based on *methacrylate* monomers are cheap to produce, easily formed and similar in color to hard tissues of teeth. Disadvantages of brackets made of polycarbonates include the material's susceptibility to discoloration, low stiffness, which has an adverse effect on treatment mechanics, loss of mechanical parameters during usage due to sorption of water and temperature change in oral cavity, as well as brittleness and susceptibility to deformation[2,3,4,5,6].

Manufacturers of orthodontic materials - in their efforts to compensate for defects of polycarbonate brackets – introduced brackets characterized by good aesthetic properties, made from high quality polyurethane or polycarbonate reinforced with non-organic fillers such as ceramics or glass fiber. These products often comprise metal slots limiting arch friction, and this solution also improved their mechanical properties[7]. However, the problem of degradation of plastic brackets in oral environment remained unsolved[8,9]. Another worrisome fact is a possible release of bisphenol A from polycarbonate plastics[10], whose potentially harmful activity on living organisms was indicated in numerous studies[11-15]. Orthodontic polycarbonate brackets are characterized by a high susceptibility to discoloration which may be caused by exposure to light and temperature changes or dyes delivered into the oral cavity with food[6,16].

Brackets made of composite materials mainly consist of polymer network of *methacrylate* or urethane monomers and of filler particles responsible for improved mechanical properties[1,17].

Good aesthetic characteristics of polycarbonate and composite brackets resulting from color matching to hard tissues of teeth and from transparency, are soon depreciated as their surface and structure become invaded by deposits of food dyes present in oral environment or by metabolites of bacterial flora. The patient's initial enthusiasm resulting from having an "invisible" appliance turns into dissatisfaction due to gradual discoloration of its most visible elements.

In mid 1980s[1,18] first ceramic brackets appeared on the market. Despite their obvious advantages, such as resistance to discoloration and good aesthetic properties, namely color similar to that of dentition and transparency allowing exposing the natural color of teeth, they were not free from defects. Unfavorable features of ceramic brackets include brittleness, fragility, increased friction between ceramics and steel as well as nickel-titanium arches (which extends orthodontic treatment time) and excessive adhesion to enamel (resulting in enamel's damage while removing brackets). Similarly to other groups of dental materials, also ceramics used in orthodontics was subjected to processes targeted at improving its properties.

Some of the above mentioned disadvantages have been eliminated. Introduction of reinforced ceramics into the production process improved brackets' mechanical resistance. Decreased friction of arches was achieved by introducing metal slots into brackets. The problem of damage of tooth's hard tissues during removal of ceramics from their surface was partly solved by resigning from chemical bonding, conditioned by the presence of monosilane on the bracket's base. Nowadays it is believed that mechanical micro-retention of the bracket's base to the bonding system provides enough bonding power to hold the element on the tooth's surface.

Aluminum oxide (Al2O3), which is the main component of contemporary orthodontic ceramics may occur in two forms: mono- and polycrystalline. Monocrystalline brackets, commonly called 'sapphire', are created by cutting the correct shape from ceramic blocks with the use of machine tools with diamond cutters[1]. Polycrystalline brackets are obtained in the process of ceramics burning in the molds which are appropriate for their shapes[1]. The two types differ in a higher homogeneity and a higher transparency of monocrystalline brackets, which allows exposition of the natural color of dentition.

An unquestionable advantage of currently used ceramic brackets is color synergism with hard tissues of teeth. The color of ceramics, which is very close to that of enamel and dentine, results in a lower visibility of appliances based on mono- and polycrystalline brackets against dental arch background compared to metal brackets, which is often decisive for the patient who is about to commence orthodontic treatment with a fixed

appliance. It is commonly believed that ceramics is resistant to staining. In oral environment conditions this feature is particularly important due to the presence of numerous chromogenic / colored compounds delivered in food and drinks[19,20,21]. On the other hand, however, the time of orthodontic treatment, which last on average two years, sets high requirements for materials from which brackets are made with regard to resistance to sorption of dyes into their structure.

Aim

The aim of the study was an assessment of susceptibility of orthodontic aesthetic brackets to staining from dyes contained in foodstuffs.

Material and methods

The study included eight types of orthodontic aesthetic brackets:

Polymer-based brackets:

1. Rave (Ortho Technology. USA) – brackets based on polycarbonates,
2. Crystal Clear Plastic Bracket (Dentsply Glenroe, USA) – brackets based on polycarbonates,
3. Silkon Plus (American Orthodontics, USA) – brackets based on polycarbonates,
4. Orthoflex (Ortho Technology, USA) – brackets based on polyurethanes.

Ceramic brackets:

5. Reflections (OrthoTechnology, USA) – polycrystalline brackets,
6. Pure (OrthoTechnology, USA) – monocrystalline brackets,
7. Contour (Class One Orthodontic Products, USA) – polycrystalline brackets,
8. Miso (HT Corp. , Korea) – monocrystalline brackets.

10 pieces of each bracket type were assessed. Before incubation of materials in foodstuff dye environment, the brackets were subjected to colorimetric analysis with the use of Spectroshade dental spectrophotometer (MHT, Italy). The assessed samples were placed on a white, matt background. The colorimetric analysis yielded numeric results describing the color of assessed aesthetic brackets in a three-dimensional coordinates space CIE L*a*b*. The scale assigns numeric values to colors of assessed objects, describing their lightness (L*) and saturation with green-red (+/-a*) and with blue-yellow (+/-b*). Then five pieces of each assessed bracket type were placed in coffee (Kraft Foods, Germany) and red wine (Sutter Home Winery Inc., USA) solutions for 24 hours. The temperature of the solutions was 20ºC. After incubation in foodstuff dye environment, the brackets were removed from solutions, rinsed with running water and cleaned with a prophylactic brush set on a low speed contra angle hand piece with a water spray. The assessed ceramic samples were subjected to another colorimetric analysis with the use of Spectroshade spectrophotometer.

The discolored orthodontic brackets are shown in Figure 1.

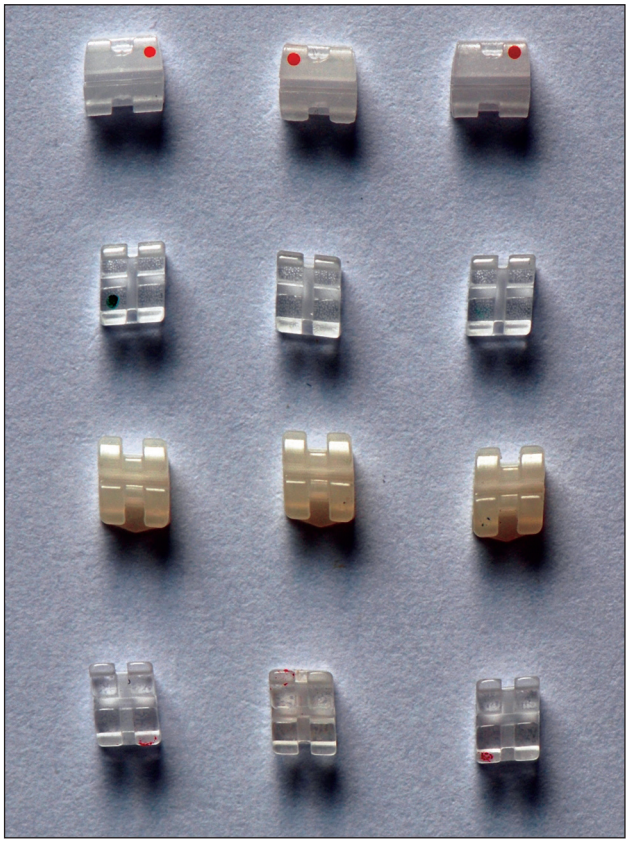

Figure 1. The discolored orthodontic aesthetic brackets

In order to obtain numeric data denoting change in individual color components of assessed brackets, i.e. ΔL*, Δa*, Δb*, differences between the parameters before and after incubation of samples in foodstuff dye environment were calcu-

lated. The total color change ΔE* was calculated with the following formula:

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$$

The obtained results were entered into MS Excel spreadsheet program and subjected to statistical analysis with the use of Statistica 6.0 software package. In order to find any correlations between the degree of discoloration and the type of assessed brackets as well as foodstuff dyes used, the two-way analysis of variance (ANOVA) was applied with NIR test at the assumed significance level of p=0.05.

Results

Total color change ΔE of brackets made of composite materials

After incubation in foodstuff dye environment, mean color change ΔE of composite brackets was 13.84 points, including 6.94 points for Rave brackets, 14.95 for Glenroe brackets, 18.19 points for Silkon brackets, and 15.28 points for Orthoflex brackets. A statistical analysis ($p<0.05$) indicated that Rave brackets demonstrated the lowest statistically significant discoloration compared to the other assessed composite brackets.

Changes in individual color parameters (ΔL, Δa, Δb, ΔE) of the assessed composite brackets are shown in Table 1.

Total color change ΔE of brackets made of ceramic materials

Mean color change ΔE of ceramic brackets after incubation in coffee and red wine solutions was 3.02 points. Mean color change in Reflections brackets was 3.78 points, in Pure brackets 2.55 points, in Contour brackets 2.11 points and in Miso brackets 3.66 points. A statistical analysis ($p<0.05$) indicated that Reflections and Miso brackets discolored to a significantly higher degree than Pure and Contour brackets.

Changes in individual color parameters (ΔL, Δa, Δb, ΔE) of the assessed ceramic brackets are shown in Table 2.

Table 1. Mean changes of numeric values describing color of polymer-based brackets after 24-hour storage in food-colorant environment

Brand	Company	ΔL	std	Δa	std	Δb	std	ΔE	std
Rave	Ortho Technology, USA	-5,45	3,76	0,88	1,50	0,87	3,32	6,94	2,93
CCPB	Dentsply Glenroe, USA	-13,02	7,85	2,62	2,18	0,79	5,72	14,95	6,87
Silkon Plus	American Orthodontics, USA	-11,01	8,80	11,12	2,94	-0,09	7,75	18,19	7,12
Orthoflex	Ortho Technology, USA	-12,66	7,47	1,38	0,66	2,42	6,60	15,28	5,20

Table 2. Mean changes of numeric values describing color of ceramic brackets after 24-hour storage in food-colorant environment

Brand	Company	ΔL	std	Δa	std	Δb	std	ΔE	std
Reflections	Ortho Technology, USA	-2,80	1,26	-0,07	0,45	1,96	1,22	3,78	0,63
Pure	Ortho Technology, USA	-1,98	1,57	0,48	0,35	1,20	0,65	2,55	1,41
Contour	Class One Orthodontic Products, USA	-1,80	0,66	0,37	0,24	0,69	0,65	2,11	0,51
Miso	HT Corp., Korea	-2,56	0,78	-0,21	0,16	2,23	1,35	3,66	0,66

Table 3. Mean changes of numeric values describing color of single-crystal and polycrystalline based brackets after 24-hour storage in food-colorant environment

mean change from baseline	single-crystal	polycrystalline	P *no significant
ΔL	-2,27	-2,30	0,9361*
Δa	0,14	0,15	0,9127*
Δb	1,72	1,33	0,2925*
ΔE	3,10	2,94	0,6507*

Color change ΔE of mono- and polycrystalline ceramic brackets

Mean total color change ΔE of polycrystalline brackets, i.e. Refelctions and Contour was 2.94 points and its magnitude was not significantly ($p>0.05$) different from the color change parameter in monocrystalline brackets, i.e. Pure and Miso, which was 3.10 points. Changes in individual color parameters (ΔL, Δa, Δb, ΔE) of the assessed ceramic brackets depending on ceramics type are shown in Table 3.

Color change of orthodontic brackets stored in coffee infusion

Lightness (L)*

Mean lightness drop in orthodontic brackets stored in coffee infusion for 24 hours was 4.23 points in case of composite brackets, and 1.49 points in case of ceramic ones.

A statistical analysis indicated that composite brackets became significantly ($p<0.05$) darker than ceramic brackets.

Red chromaticity (a)*

After incubation in coffee solution, red chromaticity in brackets made of composite materials was decreased on average by 2.55 points, whereas in the case of ceramic brackets the parameter increased by 0.07 points.

A statistical analysis indicated significant ($p<0.05$) differences in susceptibility to discoloration within the red color range of composite and ceramic brackets stored in coffee infusion.

Yellow chromaticity (b)*

Dyes contained in coffee caused an increase in mean yellow chromaticity by 6.49 points for composite brackets, and by 2.32 points for ceramic brackets. A statistical analysis indicated that within the yellow color range, composite brackets demonstrated a significantly ($p<0.05$) stronger discoloration than ceramic brackets after storage in coffee infusion.

Total color change ΔE

Mean total color change in composite brackets incubated in coffee solution equaled 8.92 points and was significantly ($p<0.05$) higher than color change in ceramic brackets, with mean value of 2.82 points.

Changes in individual color parameters (ΔL, Δa, Δb, ΔE) of the assessed orthodontic brackets stored in coffee solution are shown in Table 4.

Color change of orthodontic brackets stored in red wine

Lightness (L)*

Mean lightness drop in composite brackets stored in red wine equaled 16.84 points and was significantly ($p<0.05$) higher than lightness drop of 3.08 points in ceramic brackets.

Red chromaticity (a)*

After incubation in red wine, red chromaticity in brackets made of composite materials increased by 5.46 points. In the case of ceramic brackets the parameter increased by 0.35 points. The change was significantly ($p<0.05$) lower than in the case of brackets made of polymers.

Yellow chromaticity (b)*

Due to storage in red wine, yellow chromaticity of composite brackets was lower on average

Table 4. Mean changes of numeric values describing color of both polymer-based and ceramic brackets after 24-hour storage in cafe

Brand	Mean change from baseline after storage in cafe							
	ΔL	std	Δa	std	Δb	std	ΔE	std
Rave	-2,50	1,68	-0,12	0,23	3,88	1,23	4,91	0,94
CCPB	-5,74	1,67	0,68	0,33	6,16	1,06	8,57	1,22
Silkon Plus	-3,10	1,05	8,78	1,43	7,24	0,32	11,86	1,19
Orthoflex	-5,58	0,34	0,84	0,42	8,68	0,29	10,36	0,46
Reflections	-1,70	0,66	-0,44	0,32	3,00	0,78	3,53	0,80
Pure	-0,98	0,40	0,26	0,36	1,52	0,68	1,90	0,64
Contour	-1,28	0,49	0,18	0,18	1,26	0,32	1,82	0,55
Miso	-2,00	0,25	-0,26	0,05	3,50	0,23	4,04	0,31

by 4.50 points, ceramic brackets demonstrated a mean increase of 0.72 points. A statistical analysis confirmed statistically significant differences ($p<0.05$) with regard to color change in orthodontic brackets within the yellow color range depending on the material type of samples.

Total color change ΔE

After incubation of the assessed samples in red wine, mean total color change in composite brackets equaled 18.76 points and was significantly ($p<0.05$) higher than mean color change in ceramic brackets at 3.22 points.

Changes in individual color parameters (ΔL, Δa, Δb, ΔE) of orthodontic brackets stored in red wine are shown in Table 5.

Color change of orthodontic brackets depending on the type of foodstuff dye

In the case on brackets made of polymer materials, mean total color change ΔE after storage in coffee infusion equaled 8.92 points and was significantly ($p<0.05$) lower than color change in samples stored in red wine at 18.76 points.

Ceramic brackets stored in coffee solution demonstrated color change ΔE by 2.82 points on average, whereas after incubation in red wine this type of brackets changed color on average by 3.22 points.

A statistical analysis indicated that both beverages used in the study cause a comparable ($p>0.05$) total color change in ceramic brackets.

Discussion

A statistical analysis performed according to the described above methods indicated that color change in assessed aesthetic orthodontic brackets depends on material type from which they were made and on applied food dyes. Brackets made of polymer materials discolored to a significantly ($p<0.05$) higher degree than ceramic ones. Wine caused greater color changes in samples than coffee, irrespective of bracket types, though the difference was significant ($p<0.05$) only in the case of samples based on polymers.

Available literature reports that polymer-based dental materials used in orthodontic, conservative and prosthetic treatment are susceptible to staining with dyes present in the oral cavity. Color change caused by colorants contained in beverages, food or stimulants applies to materials used for filling cavities[18,22,23], polymers used in prosthetics[24,25,26], elastic orthodontic ligatures[27], as well as aesthetic brackets[6,28,29]

Activity of colored substances is assessed in laboratory conditions and consists in performing a colorimetric analysis before samples come into contact with colorants, incubation of brackets in solutions of coffee, tea, red wine or fruit juices, then performing another colorimetric analysis of studied dental materials. Research on color stability applies most frequently to composite materials used as cavity fillers, few studies describe susceptibility of orthodontic brackets to staining with foodstuff dyes.

In the study published by Faltermeier *et al.* [28], an impact of coffee and red wine on color change in four types of orthodontic brackets made of composite materials and polymers was assessed. For colo-

Table 5. Mean changes of numeric values describing color of both polymer-based and ceramic brackets after 24-hour storage in red wine

Brand	Mean change from baseline after storage in red wine							
	ΔL	std	Δa	std	Δb	std	ΔE	std
Rave	-8,40	2,68	1,88	1,59	-2,14	0,82	8,97	2,87
CCPB	-20,30	1,79	4,56	1,06	-4,58	0,65	21,34	1,68
Silkon Plus	-18,92	4,09	13,46	1,92	-7,42	0,93	24,52	3,52
Orthoflex	-19,74	0,34	1,92	0,29	-3,84	0,15	20,20	0,33
Reflections	-3,90	0,29	0,30	0,12	0,92	0,15	4,02	0,30
Pure	-2,98	1,70	0,70	0,12	0,88	0,48	3,20	1,73
Contour	-2,32	0,28	0,56	0,09	0,12	0,18	2,40	0,29
Miso	-3,12	0,73	-0,16	0,22	0,96	0,15	3,28	0,72

rimetric measurements the authors used a Minolta CM-C3500 (Minolta, Japan) spectrophotometer. Faltermeier *et al.*[28] studied the following brackets: Aesthetic-Line (Forestadent, Germany), Brillant (Forestadent, Germany), Envision (Ortho Organizers, USA), and Exper – experimental polyurethane-based brackets. After 24-hour incubation the authors described total color change ΔE of samples, which ranged from 1.93 to 10.41 points for coffee, and from 0.33 to 10.15 for red wine.

The results of the current study, describing color change in polymer-based orthodontic brackets are close to the values reported by Faltermeier *et al.* [28] for composite-based brackets. Although in the two studies the authors assessed different bracket types and applied a different methodology for color change registration, the results of both studies prove that polymer brackets are highly susceptible to absorption of foodstuff dyes. An assessment of color change in orthodontic brackets due to exposure to food colorants was also described by Wriedt *et al*.[29]. The quoted authors assessed the impact of UV radiation, orange juice, red wine, coffee and tea on color stability in six types of orthodontic brackets. The authors studied the following ceramic brackets: Fascination 2 (Dentaurum, Germany), Ceramic 20/40 M (American Orthodontics, USA), Mystique (GAC, USA), and polymer-based brackets: Aesthetik-Line (Forestadent, Germany), Brillant (Forestadent, Germany), Silkon M (American Orthodontics, USA). For colorimetric assessment the authors used an EasyShade (VITA GmBH, Germany) spectrophotometer. After 24-hour incubation in black tea solution, mean total color change ΔE reported by Wriedt *et al*.[29] ranged from 7.7 to 11.9 points for brackets made of composite materials, and from 6.7 to 9.9 for ceramic brackets. The values of total color change in polymer-based aesthetic brackets reported by Wriedt *et al*.[29] are comparable to values obtained in the current study for brackets made of composite materials. In the case of ceramic brackets, the quoted authors reported the brackets' susceptibility to staining which was many times higher compared to that observed in the current study. Unfortunately, a direct comparison of the results of both studies is not possible due to different methods of colorimetric analysis and different types of brackets being assessed. The quoted authors in their publication[29] also did not provide any results for samples stored in coffee solution and in red wine, which makes the comparison even more difficult. The results of this study and studies of other authors indicate a susceptibility of aesthetic orthodontic brackets to discoloration by substances included in beverages, food and stimulants.

It is a commonly known fact that polymer-based materials used in conservative dentistry, prosthetics and orthodontics are not completely chemically stable in oral cavity environment, where they undergo constant degradation by mastication forces, temperature fluctuations, presence of water and changes of pH[30-33]. Structure inhomogeneity, which is characteristic for polymer materials [34] can also be conducive to absorption of colorants. Therefore it is not surprising that color changes in polymer-based elastic brackets observed in this study were considerable.

It is commonly believed that ceramic orthodontic brackets are resistant to the activity of food colorants delivered into oral cavity environment with meals, beverages and stimulants. The results of this study and the study by Wriedt *et al*. [29], while not confirming this argument, indicate a necessity of further research in order to determine their susceptibility to discoloration.

The color stability of aesthetic brackets does not depend solely on dye adsorption from the surrounding environment. The study by Lee[16] demonstrated that they also change color with temperature fluctuations. After 5,000 cycles of thermal ageing, the quoted author observed color changes in samples within the range from 1.4 points to 6.4 points, depending on the type of assessed bracket.

The results of this study and the results reported by the quoted authors confirm that aesthetic orthodontic brackets are susceptible to absorption of colorants from foodstuffs, which may have a negative impact on their visual qualities during treatment. In the case of dental materials used in oral cavity, a total color change ΔE above three points is considered unacceptable from the aesthetic point of view[26,35,36].

In this study, a mean total color change ΔE in all brackets made of composite materials and in two out of four assessed ceramic brackets, was above three points, exceeding the limit described as a boundary value for the aesthetics of dental

materials. Naturally, the results of laboratory studies cannot be directly applied to the oral cavity environment, however they constitute a basis for comparing the functional properties of individual types of orthodontic brackets and for verification of the thesis that ceramics used for production of orthodontic brackets is an ideal material with regard to aesthetic value.

Conclusions

In the conditions of this study, both polymer and ceramic orthodontic brackets demonstrate susceptibility to discoloration with dyes from foodstuffs.

The intensity of color change in ceramic brackets depends both on material type from which they are made and on the applied food colorant.

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Prevalence and correlates of school violence and sexual abuse among adolescents in Tokat, Turkey

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Abstract

The aims of this cross-sectional study were to determine the prevalence and correlates of school violence and sexual abuse among adolescents in Tokat-Turkey. The study is based on a sample of 5032 students in grade 6-8 in all schools of Tokat city in Turkey. The instrument used in the study was a Turkish translation of the ICAST-C developed by SPCAN. Students were subjected to physical (57.0%), psychological (59.8%), and sexual (6.4%) abuse with varying frequencies. A large majority (73.4%) of students experienced one or more of the types of violence. All types of violent behaviors were more common among male than female students and were experienced significantly more frequently in urban areas than in villages or districts. To address violence against students, we must first conduct nationwide studies to provide a more definitive picture of incidence and prevalence rates and to clarify relevant risk and protective factors. Second, to ensure the accuracy of the data, specific guidelines and questionnaires are needed. Third, the deleterious consequences of violence must be explained to achieve greater public awareness about victimization in schools. Fourth, the authorities must ensure and reinforce the safety and rights of children by enacting strict laws against violence and abuse.

Key words: School violence, abuse, physical violence, sexual abuse, psychological violence.

Introduction

The history of humankind has been characterized by multiple and complex forms of individual and societal violence. This complexity renders it difficult to determine the cause(s) of violence (1). Although violence among children and adolescents is a serious problem throughout much of the world, the prevalence of various types of violence against children and adolescents remains unclear (2,3) despite data showing that children are victims of crime about twice as frequently than are adults over 25 years of age (4). The term "violence" refers to a destructive physical act that is performed to hurt or morally degrade another human being (5,6). Child abuse is defined as all kinds of violence against a child and is evaluated within a multidisciplinary perspective (medical, legal, psychological, social) (7). No clear definition of school violence exists, but it can be narrowly defined as synonymous with physical violence perpetrated by school-age children within the school setting (8). Most school violence can be categorized as 1) physical coercion or physical injury, 2) verbal aggression and mental cruelty, or 3) bullying (9). In schools, sexual abuse and violence are inextricably linked to other forms of physical violence (10).

Violence and childhood physical and sexual abuse in schools are increasing at an alarming rate worldwide (11), including in Turkey; however, the current status of child abuse in our country remains unknown. Contrary to popular belief, school violence is an important problem in many regions of the world, affecting both developing and industrialized countries as well as rural and urban areas (11) . Since the 1980s, much research has been conducted in Western countries to investigate childhood physical and sexual abuse. Nonetheless, few studies have been conducted in Turkey (7), and our official statistical reports are not as advanced as those in Western countries that require physicians to report violence and child

abuse. Indeed, much of the world does not have such an advanced reporting system. Both underreporting and cultural differences may affect the reported rates of child abuse and neglect (3).

Schools should be safe places for children and adolescents. Although schools are secondary socialization places, ranking after the home in this regard, violence that starts at home may continue at school (12, 13). Unfortunately, increasing violence among youths is a serious public health problem that must be addressed. Exposure to any kind of violence has detrimental consequences including academic problems, aggressive behavior, and somatic complaints (14). These unfavorable consequences can have both short- and long-term effects.

Additionally, victims (students) may be socialized (15) to consider violence to be legitimate and go on to perpetrate violence on other students and peers (16). Youths spend most of their time at school, where they can be exposed to violence perpetrated by their peers or by school employees. Before implementing programs aimed at preventing school violence and child physical and sexual abuse, authorities must ascertain the prevalence of these phenomena. As noted above, countries also have differing attitudes about child abuse; thus, each country must develop its own questionnaires to identify abuse at school or in the workplace.

This study examined the prevalence of school violence and of physical and sexual abuse in schools. We also sought to determine the sociodemographic characteristics of victimized adolescents in Turkey.

Methods

This study focused on students attending the sixth, seventh, and eighth grades of middle schools in downtown Tokat and its surrounding villages during the 2010–2011 academic year. The number of students in these grades was obtained from the Tokat National Training and Education Directorate. In total, 33,573 students were enrolled in these grades, and our sample included 5,031 students. More specifically, we used clustered sampling based on the locations of the schools in the city, towns, and villages and stratified sampling according to grade to select 15% of the total population to participate in this study.

The instrument used in the study was a Turkish translation of the International Child Abuse Screening Tool-children's version (ICAST-C) developed by the International Society for the Prevention of Child Abuse and Neglect (ISPCAN). The Turkish version was prepared according to relevant expert opinions and related publications. The questionnaire collected data on the sociodemographic characteristics of the students, their family structure, the presence and type(s) of abuse directed at the children at school, and the identity of the abuser(s).

Written informed consent was obtained from the students and their families prior to the completion of the questionnaire. A preliminary survey was conducted among 350 students, and the results were evaluated. The survey was performed under the supervision of the school counselor, and students were reminded about the confidentiality of the information provided; all forms were distributed and collected following a double-blind procedure.

Data from 5,031 student volunteers were analyzed. Questionnaires were excluded from the analysis because of inconsistent responses or a substantial proportion of missing data; 5,025 questionnaires were included in the analyses. SPSS for Windows software was used to analyze all data. Statistical analyses were performed using the *chi*-squared test, and statistical significance was set at $p<0.05$.

Results

Of the students who participated, 61.2% always felt safe at school, 23% generally felt safe at school. 11.9% occasionally felt safe at school, and 3.5% never felt safe at school.

Table 1 shows that participants experienced being subjected to pain (47.9%), being slapped in the face or head (34.3%), being hit on the hand or arm (28.9%), having an ear pulled (22.5%), being forced to stand or kneel (16.5%), and having their hair pulled (14.9%).

Table 2 shows that the following types of psychological violence were experienced by participants: being sworn at (41.6%); having items stolen, broken, or sullied in some way (27.3%); being the target of efforts to humiliate, degrade, or embarrass (24.5%); being called a profane or offensive nickname (24.5%); and deliberately being made a fool of (15.6 %).

Table 1. Types of physical violence directed at students, and their perpetrators

Questions	Answered	Unspecified		Adults		Students		Both		Total	
	n	n	%	n	%	n	%	n	%	n	%
Hurt you or caused pain to you at school	4949	371	15,6	323	13,6	1423	60,0	255	10,8	2372	47,9
Slap you with a hand on your face or head as punishment?	4926	302	17,9	878	52,0	357	21,1	152	9,0	1689	34,3
Slapped you with a hand on your are mor-hand?	4937	91	6,4	884	62,0	338	23,7	113	7,9	1426	28,9
Twisted your ear as punishment?	4747	85	8,0	835	78,2	99	9,3	49	4,6	1068	22,5
Pulled your hair as punishment?	4580	45	6,6	426	62,6	165	24,2	45	6,6	681	14,9
Hit you by throwing an object at you?	4991	72	9,2	200	25,6	469	60,0	41	5,2	782	15,7
Hit you with a closed fist?	4896	44	10,2	74	17,2	284	66,0	28	6,5	430	8,8
Kicked you?	4652	44	10,5	105	25,0	247	58,8	24	5,7	420	9,0
Crushed your fingers or hands as punish-ment?	4545	8	5,2	75	48,4	58	37,4	14	9,0	155	3,4
Putsome thing like pepper in your mouth	4946	32	37,6	35	41,2	13	15,3	5	5,9	85	1,7
Made you stand /kneel in a way that hurt stopunish you?	4955	96	11,7	642	78,3	55	6,7	27	3,3	820	16,5
Took your food away from you as punish-ment?	4997	31	15,7	95	48,0	66	33,3	6	3,0	198	4,0
Forced you to do some thing that was dan-gerous?	5001	13	10,4	32	25,6	70	56,0	10	8,0	125	2,5
Choked you?	4994	12	16,9	19	26,8	35	49,3	5	7,0	71	1,4
Tied you up with a rope or belt at school	5002	6	16,2	12	32,4	18	48,6	1	2,7	37	0,7
Tried to cut you purpose fully with a sharp object?	4911	28	27,7	14	13,9	50	49,5	9	8,9	101	2,1

Table 2. Types of psychological violence directed at students, and their perpetrators

Questions	Answered	Unspecified		Adults		Students		Both		Total	
	n	n	%	n	%	n	%	n	%	n	%
Sworn at you?	5000	131	6,3	93	4,5	1669	80,3	186	8,9	2079	41,6
Deliberately in sulted you?	4995	40	7,8	57	11,2	366	71,6	48	9,4	511	10,2
Shouted at you to embarrass or humiliate you?	4982	76	6,2	447	36,5	503	41,1	197	16,1	1223	24,5
Called you rude or hurtful names?	4994	63	5,9	142	13,3	773	72,3	91	8,5	1069	21,4
Purposely made you feel stupid or foolish?	4996	66	8,5	202	25,9	409	52,4	103	13,2	780	15,6
Referred to any health problems you might have in a hurtful way?	5003	11	3,2	49	14,1	250	71,8	38	10,9	348	7,0
Stopped you from being with other children to make you feel bad or lonely?	4995	41	12,0	189	55,4	88	25,8	23	6,7	341	6,8
Tried to embarrass you because you were an or phan or without a parent?	4984	23	29,1	14	17,7	36	45,6	6	7,6	79	1,6
Embarrassed you because you were poor or unableto buy things?	5001	13	8,0	31	19,0	108	66,3	11	6,7	163	3,3
Stole or broke or ruined your belongings?	4984	172	12,6	23	1,7	1118	82,1	49	3,6	1362	27,3
Threatened you with bad Marks that you didn't deserve?	4994	53	11,6	36	7,9	346	75,9	21	4,6	456	9,1

Table 3. Types of sexual abuse directed at students, and their perpetrators

Questions	Answered	Unspecified		Adults		Students		Both		Total	
	n	n	%	n	%	n	%	n	%	n	%
Touched your body in a sexual way or in a way that made you uncomfortable?	4529	7	4,9	18	12,7	110	77,5	7	4,9	142	3,1
Showed you pictures, magazines, or movies of people or children doing sexual things?	4466	4	2,9	28	20,1	99	71,2	8	5,8	139	3,1
Made you take your clothe soff when it was not for a medical reason?	4462	0	0,0	6	28,6	10	47,6	5	23,8	21	0,5
Opened or took their own clothe soff in front of you when they should not have done so?	4468	6	16,7	7	19,4	20	55,6	3	8,3	36	0,8
Did anyone at school make you have sex with them?	4459	2	4,3	2	4,3	40	87,0	2	4,3	46	1,0
Did anyone at school make you touch their private parts when you didn't want to?	4470	2	2,8	9	12,5	53	73,6	8	11,1	72	1,6
Did anyone at school give you money/ things to do sexual things?	4455	3	13,6	6	27,3	11	50,0	2	9,1	22	0,5
Did anyone at school involve you in making sexual pictures or videos?	4469	8	29,6	8	29,6	8	29,6	3	11,1	27	0,6
Did anyone at school kiss you when you didn't want to be kissed?	4966	6	9,2	5	7,7	51	78,5	3	4,6	65	1,3

Table 3 shows the types of sexual abuse experienced by participants, including being touched for sexual purposes (3.1%); being exposed to pornographic pictures, magazines, or videos (3.1%); having intimate body parts touched (1.6%); and being kissed against their will (1.3%).

This study revealed that psychological violence was the most prevalent type of abuse, and that physical violence was usually perpetrated by adults, whereas sexual abuse was usually perpetrated by other students.

As shown by Table 4, physical violence was most common among boys (19.6%), whereas emotional violence (29.2%) was most common among girls. Both male and female students were victims of concomitant physical and emotional violence, although combined physical and sexual abuse was the least (0.1%) common type of abuse.

As seen in Table 5, students were subjected to to physical violence (57.0%), emotional violence (59.8%), and sexual (6.4%) abuse with varying frequencies. A large majority (73.4%) of students experienced one or more of the types of violence. All types of violent behaviors were more common among male than female students and were experienced significantly more frequently in urban areas than in villages or districts. The results revealed a significant increase in the incidence of all types of violence as children advanced in school. A significant correlation between the educational level of the mother and experience with violence was also noted. Specifically, children of mothers who graduated from high school or university experienced less violence. Variable correlations were observed between the educational level of the father and the severity and frequency of experiences with violence. Children of fathers with a high school or university education were less likely (54.4%) to experience physical violence, whereas children of illiterate fathers were less likely (56.0%) to become victims of emotional violence. However, sexual violence was directed primarily (14.9%) against children of illiterate fathers. The profession of mothers appeared to be related to the incidence of children's experiences with violence. None of the students who defined their mothers' job as "shopkeeper" was a victim of sexual abuse, and the incidence of sexual abuse was relatively low (3.7%). among children of mothers working as civil servants. Physical (33.3%) and emotional (55.6%) violence were experienced relatively less frequently by children of female pensioners. When the correlation between father's job and type of violence was examined, minimal rates of physical, emotional violence, and sexual abuse were observed among children whose fathers were civil servants, farmers, and tradesman, respectively. Experience with sexual violence occurred less frequently among children whose fathers or mothers were shopkeepers.

No significant difference was found between number of siblings and types of physical violence or sexual abuse, whereas a significant difference was detected with respect to emotional violence. Interestingly, emotional violence was most common (63.6%) in families with a single child, whereas it was relatively lower (55.4%) among children with ≥5 siblings. On the other hand,, the incidence of sexual abuse was lowest (3.3%) in

Table 4. Distribution of the types of violence according to gender of the students

	Boys		Girls		Total	
	Number	%	Number	%	Number	%
Physical	393	19,6	272	16,2	665	18,0
Emotional	281	14,0	490	29,2	771	20,9
Sexual abuse	9	0,4	5	0,3	14	0,4
Physical + Emotional	1131	56,4	799	47,6	1930	52,4
Physical +Sexual abuse	2	0,1	1	0,1	3	0,1
Emotional + Sexual abuse	9	0,4	27	1,6	36	1,0
Physical + Emotional + Sexual abuse	182	9,1	85	5,1	267	7,2
Abused children Total	2007	78,2	1679	68,3	3686	73,4
Unabused children	559	21,8	780	31,7	1339	26,6

X^2=215,8, p< 0,0001

Table 5. Distribution of types of violence according to the sociodemographic characteristics of the students

Gender of the students (n=5025)	n	%	Physical violence (%)	Emotional violence (%)	Sexual abuse (%)	Any other type of violence (%)
Boys	2566	51.1	66.6	62.5	7.9	78.2
Girls	2459	48.9	47.1	57.0	4.8	68.3
			X^2=195. p<0.0001	X^2=15.8. p<0.0001	X^2=19.9. p<0.0001	X^2=63.4. p<0.0001
Location of the school where exploitation occurred (n=5025)	**n**	**%**	**Physical violence (%)**	**Emotional violence (%)**	**Sexual abuse (%)**	**Any other type of violence (%)**
City center	1324	26.3	54.5	64.1	7.6	73.8
Town center	1644	32.7	62.0	64.2	8.3	77.1
	2057	40.9	54.6	53.5	4.0	70.1
			X^2=25.1. p<0.0001	X^2=57.6. p<0.0001	X^2=32.5. p<0.0001	X^2=23.6. p<0.0001
Grade of the students (n=5025)	**n**	**%**	**Physical violence (%)**	**Emotional violence (%)**	**Sexual abuse (%)**	**Any other type of violence (%)**
6	1579	31.4	54.4	59.6	5.9	72.1
7	1768	35.2	56.4	56.1	6.0	70.5
8	1678	33.4	60.2	64.1	7.2	77.5
			X^2=11.5. p=0.003	X^2=22.7. p<0.0001	X^2=2.7. p=0.3	X^2=23.5. p<0.0001
Educational status of the mother of the student exposed to violence (n=4928)	**n**	**%**	**Physical violence (%)**	**Emotional violence (%)**	**Sexual abuse (%)**	**Any other type of violence (%)**
Illiterate	467	9.5	54.7	59.7	2.8	67.5
Primary school	3348	67.9	57.3	59.3	6.7	73.0
Secondary school	572	11.6	57.7	62.4	8.6	74.5
Lycée	447	9.1	56.8	64.0	5.8	76.7
High school- University	94	1.9	35.1	39.8	3.2	41.9
			X^2=19.5. p<0.001	X^2=20.7. p<0.001	X^2=16.7. p=0.002	X^2=54.9. p<0.0001
Educational status of the father of the student exposed to violence (n=4940)	**n**	**%**	**Physical violence (%)**	**Emotional violence (%)**	**Sexual abuse (%)**	**Any other type of violence (%)**
Illiterate	144	2.9	62.5	56.0	14.9	72.3
Primary school	2737	55.4	57.1	58.3	5.3	72.5
Secondary school	803	16.3	58.5	62.6	8.0	75.2
Lycée	925	18.7	54.7	63.8	7.5	75.8
High school University	331	6.7	54.4	59.2	5.7	63.7
			X^2=5.4. p=0.2	X^2=12.2. p=0.02	X^2=28. p<0.0001	X^2=20.7. p=0.0009
Mother's job (n=4970)	**n**	**%**	**Physical violence (%)**	**Emotional violence (%)**	**Sexual abuse (%)**	**Any other type of violence (%)**
Civil servant	82	1.6	58.5	67.1	3.7	76.8
Worker	152	3.1	60.7	67.3	10.7	77.3
Shopkeeper	23	0.5	43.5	65.2	0.0	69.6
Farmer	13	0.3	61.5	61.5	7.7	74.6
Pensioner	7	0.1	33.3	55.6	11.1	55.6
Housewife	4693	94.4	57.2	59.2	6.3	63.6
			X^2=116.8. p<0.0001	X^2=6.3. p=0.2	X^2=11.7. p=0.07	X^2=20.5. p<0.001

Father's job(n=4766)	n	%	Physical violence (%)	Emotional violence (%)	Sexual abuse (%)	Any other type of violence (%)
Civil servant	560	11.7	54.1	62.9	6.1	74.1
Worker	1199	25.2	57.9	63.7	7.4	77.4
Shopkeeper	427	9.0	55.7	61.1	5.2	74.5
Farmer	1144	24.0	52.7	52.3	5.9	67.0
Self employed	503	10.6	65.6	67.4	7.8	78.5
Pensioner	121	2.5	54.5	60.3	5.8	74.4
Unemployed	812	17.0	63.3	67.8	6.8	73.4
			X^2=39.1. p<0.0001	X^2=65.3. p<0.0001	X^2=5.3. p=0.5	X^2=40.8. p<0.0001
Number of siblings(n=4778)	**n**	**%**	**Physical violence (%)**	**Emotional violence (%)**	**Sexual abuse (%)**	**Any other type of violence (%)**
1	151	3.2	55.6	63.6	3.3	75.5
2	1072	22.4	57.3	63.3	6.8	75.2
3	1613	33.8	57.2	59.9	6.0	73.9
4	983	20.6	55.8	57.4	6.7	70.5
≥ 5	959	20.1	55.3	55.4	7.8	71.1
			X^2=0.9. p=0.9	X^2=16.3. p=0.003	X^2=5.9. p=0.2	X^2=8.6. p=0.07
Monthly income of the family (n=4543)	**n**	**%**	**Physical violence (%)**	**Emotional violence (%)**	**Sexual abuse (%)**	**Any other type of violence (%)**
<650 TL	2216	48.8	54.8	54.4	5.7	69.2
651-1350 TL	1491	32.8	58.6	64.6	7.0	77.1
1351-1900 TL	554	12.2	55.8	64.3	7.4	77.4
≥ 1900 TL	282	6.2	61.4	67.5	8.2	79.3
			X^2=8.1. p=0.04	X^2=52.4. p<0.0001	X^2=5.0. p=0.2	X^2=40.6. p<0.0001
Number of children exposed to violence(%)			57.0	59.8	6.4	73.4

families with a single child, whereas it was highest (7.8%) among children with ≥5 siblings. Interestingly, children in families with higher incomes were more likely to experience physical, emotional, and sexual abuse, whereas these were rarely encountered in families with lower incomes.

Discussion

To our knowledge, this is the first published study to assess physical, psychological violence, and sexual abuse in a sample of adolescents at schools in Turkey. The study was conducted at schools in both rural and urban areas around Tokat city. We believe that our study sample reflects the Turkish population of adolescents.

The results of the present study revealed that 73.4% of the sample had experienced at least one kind of violence at school was, and that the prevalence of physical abuse varied by sex. Physical violence was experienced more frequently by the boys than by the girls in our sample. Our findings were consistent with the literature showing that males are more likely to be exposed to physical violence and to be subjected to physical punishment and discipline (17). Although previous studies have reported that the perpetrators of physical violence were generally male staff or male peers (18), we did not ask whether the perpetrator was male or female, precluding comparisons of the sex of perpetrators.

Our data revealed that 0.4% of the sample had experienced sexual abuse; this is very low compared with other reports in the literature (3, 18, 19). The prevalence of both physical and sexual abuse was 0.1% and that of both psychological and sexual abuse (combined violence) was 1%.

All three kind of abuse were reported by 7.2% of the sample, and 8.7% reported sexual abuse. Reported sexual victimization rates have ranged between 8% and 48% in different countries, and our results were consistent with this literature (3).

Many studies have reported higher rates of sexual abuse among girls than boys (20,21) The present study revealed no sex differences in the rates of sexual abuse among students attending Tokat schools. However, the rates of physical and psychological violence differed according to the jobs of fathers. According to a substantial body of research, unemployment affects the prevalence of violence and abuse in that the children of unemployed fathers were more likely to have been exposed to physical and psychological violence than were children of employed fathers (22).

A recent study comparing preschool children, primary-school children and adolescents found that the prevalence of sexual abuse may increase with age, whereas that of physical violence may decrease with age (23). Although our sample was drawn from middle schools, precluding comparisons among a wide range of age groups, we found no significant differences in sexual abuse according to age, and physical and psychological violence were more common in higher grades. When students get older, they may be subjected to more punitive disciplinary measures because school staff may feel they need recourse to physical means when older students fight and argue with their peers.

Psychological violence was the most common type of violence experienced by our sample (59.8%). Psychological violence can occur anywhere, and it has been reported to occur frequently in schools (24, 25). Despite its high prevalence, psychological violence involving children is underreported (26) because of the difficulty in defining psychological violence and because the abusers are typically teachers or friends. Indeed, most children are not comfortable reporting a teacher or friend.

Although corporal punishment (physical violence) at schools is forbidden by law in Turkey, as in many other countries, physical violence does nonetheless occur in these situations and is regarded as a kind of discipline rather as abusive if it is performed by teachers (18,19). The UNICEF study "A Gender Review in Education" reported that physical abuse was more common in boys in Turkey, who were more likely to be exposed to corporal punishment for disciplinary purposes (27). Proverbs such as "The fact that a mother/teacher beats her child/student leads him/her to obedience and good deeds (roses grow everywhere on the body of a child beaten by his/her mother/teacher)" and "You can teach my child however you want (the meat of my child is yours and bones are mine)" describe the attitudes of many parents toward their child's education and the status of teachers. Sociologists have suggested that violence should be addressed within the dynamics of social relationships given that violence typically occurs between related individuals and groups. In this context, the particular types of violent behaviors considered to represent child abuse are closely related to the social structure, culture, and values of each society (1).

We found not sex difference in experiences with both physical and psychological violence, but girls were more likely to be subjected to psychological violence alone. Half our population had experienced both physical and psychological violence.

The perpetrators of the physical violence that occurs at schools are primarily adults, especially teachers and principals. School staff used corporal punishment such as slapping, twisting ears, pulling hair, crushing fingers, and forcing the student to stand or kneel, but more dangerous forms of physical violence (e.g., punching, kicking, hitting with an object, and using a sharp object to inflict cuts) were perpetrated by other students.

Teachers play important roles in protecting children from harmful events and from victimization and in providing safe places in school (18). Unfortunately, in some cases, adults, especially teachers, perpetrate emotional, physical violence, and sexual abuse on their students (28).

According to the literature, children who are from minority and poor families are victimized more frequently than are others (14, 19, 22, 28). Our subjects from villages were less likely to have experienced psychological and physical violence than were those who lived in urban areas. This difference may be attributable to the status of towns, which is between villages and cities and thus neither completely rural nor completely urban in terms of structure and values. We did not find any differences in the rates of sexual abuse according to family income. In Taiwan, sexual abuse was

more common in low-income families, and perpetrators often attracted the child by offering food, money, or material comfort. Another explanation is that rural areas have fewer educational and medical services available than do urban areas, rendering the reporting of abuse more difficult (19). Afifi et al. showed that abuse was not significantly associated with the income or property ownership of the family, but that crowding was important in predicting emotional abuse (23). In contrast to the results reported by Afifi et al., our data showed that psychological violence was more prevalent in single-child families (23).

We found that the rates of sexual abuse were lower among educated and highly educated parents and that no association existed between paternal educational level and physical violence, but that psychological violence was directed primarily at the children of educated fathers. Parental level of education has been associated with patterns of teaching, guiding, stimulating, and communicating with children (29). The more closely guided and highly motivated children of more educated fathers have been found to be more likely than the less closely guided and motivated children of uneducated or less educated fathers to perceive any given behavior as violent (29).

Bullying, one kind of violence, is an increasing problem in many countries and in Turkey in particular. This practice involves a conscious desire to hurt, threaten, or frighten someone else or the use of aggression with the intent to hurt another person (30).

A more detailed examination of our results revealed that other students or peers were the perpetrators of bullying. Few studies have been conducted about bullying or violence in Turkish schools, but the perpetrators of sexual abuse and psychological violence were other students in more than 50% of the cases identified by this study. The higher incidence of cases of sexual abuse perpetrated by school children may be explained by considering that students experience maturational processes in the context of inadequate sex education. Thus, satisfactory education and training regarding pubertal changes and sexuality may be beneficial and should be provided for students. Additionally, training about protection from any type of exploitation should be mandatory. These services should be provided by schools and psychological counselors in accord with relevant requirements.

The collection of information about sexual violence or abuse presents a major challenge to researchers because these practices are commonly considered shameful and thus remain hidden. Questions about physical and psychological violence in our study were answered by about 95%–98% of the study participants, but at least 500 students (10%) did not answer the questions about sexual violence or abuse, perhaps because they had experienced sexual abuse or violence and did not want to acknowledge these events.

Given our large sample size, the findings of this study should serve as a source of information for authorities, parents, and educators with respect to the types of child abuse that occur at schools. From this perspective, educational consultants and psychological counselors should play more important roles in the establishment and development of a welcoming psychological climate. Indeed, the management practices of schools are as important as their guidance and counseling services in efforts to prevent or at least reduce the incidence of abuse.

Psychological violence seems to be related to communication and social skills, whereas physical abuse can be regarded as associated with anger management and stress tolerance. School counseling services and psychological counselors should seek to enhance their abilities to tolerate stress and anxiety, manage anger, and resolve conflicts and problems.

Another study was conducted in the schools in Mersin, a Turkish city with a population of 4,143 students, but this research examined only physical and verbal violence. According to the results, 51.7% of the participants reported experiences with verbal violence and 38.9% reported experiences with physical violence. Boys were at higher risk than girls for both verbal and physical violence. Our results are consistent with those of this previous study (31).

A study conducted in Ankara, the capital of Turkey, revealed that 33.5% of the sample reported experiences involving physical abuse, 35.3% reported experiences involving verbal abuse, 28% reported experiences involving emotional abuse, and 15.6% reported experiences involving sexual

bullying (32). This study examined only bullying by peers, and violence perpetrated by educators and other adults was not considered.

The present study has some limitations. The sample consisted of students in Tokat, which might limit the generalizability of our results. Moreover, because the questionnaires were self-administered, social desirability may have affected the results, leading to an underreporting of less acceptable experiences, including those involving sexual abuse.

In conclusion, violence in schools is an important public health problem. Physical and psychological violence are common occurrences in schools in Turkey, and we need to increase public awareness about the seriousness of child victimization at the hands of teachers and peers in school settings. To address violence against students, we must first conduct nationwide studies to provide a more definitive picture of incidence and prevalence rates and to clarify relevant risk and protective factors. Second, to ensure the accuracy of the data, specific guidelines and questionnaires are needed, as was the case in our study. Because cultural differences can influence perceptions about violence, appropriate techniques must be used to obtain more accurate data about violence in schools. Third, the deleterious consequences of violence must be explained to achieve greater public awareness about victimization in schools. Fourth, the authorities must ensure and reinforce the safety and rights of children by enacting strict laws against violence and abuse.

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Prevalence of Group B Streptococci (GBS) colonization in pregnant women and their infants' outcome in Yazd, Iran

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Abstract

Introduction: Group B streptococci (GBS) are gram positive cocci which may cause sepsis in newborns, however pregnant woman colonized with GBS may be asymptomatic. Colonization of the newborns with GBS through pregnant mothers who carries GBS may lead to the early sepsis of newborns, pneumonia, prematurity and consequently increasing in mortality. According to the current guidelines and defined risk factors, pregnant women who are carriers of GBS are treated by antibiotics commonly by penicillin.

Method: In this descriptive-analytical study, 331 vaginal specimens of pregnant women with gestational age of ≥34 weeks were examined for GBS and culture results were obtained. Data about gestational age, delivery method as well as newborns' records such as head circumference, birth weight and height, apgar score and anomalies were also gathered and analyzed.

Results: Out of 331 cases, 57 (17.2%) were positive for GBS. Among positive cases, 29(21.8%), 25(14%) and 1(6.7%) was seen in age groups of 15-24, 25-34 and ≥35 years respectively.

There was no significant difference between both positive and negative GBS colonization groups in terms of mean gestational age, head circumference, birth weight, birth height and hospitalization period.

Conclusion: Irrespective of high prevalence of GBS colonization in pregnant women, there was no significant difference in neonatal outcome in both positive and negative GBS carrier groups.

Key words: GBS colonization, pregnant mother, newborn.

Introduction

Group B streptococci (GBS) has been known as a risk factor for preterm delivery because of high tendency to colonize in the genital tract of the pregnant women. GBS is a gram positive bacteria having the capacity to colonize in human digestive system and in women's genital tract. The presence of GBS colonization at the later gestational period in women's reproductive tract can cause serious infections in mothers and infants (1). In pregnant women who have had vaginal delivery, GBS colonization has led to a higher number of PROM* cases, postpartum fever and endometritis (2,3). GBS can causes urinary tract infection, corioamnionitis, endometritis and septicemia in pregnant women (4).

Vaginal colorization with GBS has been reported between 20-30% of cases of pregnant women in multiple studies. Approximately 60% of infants born from infected mothers with GBS remain colonized. Other risk factors which contribute to GBS colonization in infants are preterm delivery, PROM and fever at birth. Early-onset sepsis in newborns is a complication resulting from GBS colonization in pregnancy(5).

Methods

This is a cross-sectional study in which all pregnant women who were visited and delivered at Dr. Mojibian private hospital between Jan. 2008 to Feb. 2011 were studied .Vaginal secretions was searched for detection of GBS using both culture and direct gram stain. A questionnaire was completed for each patient including the gestational age, delivery type

and data related to their infants such as head circumference, birth weight and height, apgar score, neonatal death, anomalies and admission in NICU.

PROM*: Prolonged Rupture Of Membrane

Specimens from pregnant women with the gestational age of ≥34 weeks were taken and prepared by trained general practitioner and were carried to the laboratory in an hour. The samples were taken from the foreshet, perineum and anus with 2 sterile swaps. Swaps were placed in a sterile tube and were transferred to the laboratory for gram stain and cultural technique.

One of the swaps was smeared on a slide for gram stain, the bacteria were studied and the results were recorded. The other swap was inoculated on a blood agar containing 5% sheep blood and was incubated at 37^0c for 24 hours.

Smearing process was accomplished in a way to spread all the bacteria homogenously so as to help isolate growth of each colony and thus facilitate investigation.

After 24-hour incubation at 37 ^{o}C, the grown colonies were examined and tiny and mucoid colonies with beta-hemolysis were selected. Catalase tests and other necessary tests were also done. Final diagnosis was achieved by inoculating a suspect colony in a liquid hyporate medium. Following 4-5 hr. incubation, appearance of violet color revealed GBS positive.

If the test was positive, the patient was identified as GBS carrier and was treated by ampicillin during delivery period.

By setting α=5%, GBS prevalence of about 15%, marginal error d=4% and 10% missing, the sample size of 331 was selected. Data about incomplete questionnaires for mother or newborn records was excluded.

Data were analyzed using SPSS software version 16 and analyzed by independent t-test, chi square and Mann Whitney tests. Significance was set at $P \leq 0.05$.

Results

Mothers' outcome

The results indicated that out of 331 pregnant women, 57 (17.2%) were positive colonization for GBS (table1). Out of positive GBS cases, 20 had cesarean section and the rest had vaginal delivery. Out of patients with vaginal delivery, 94 cases had negative GBS. There was no hypertensive case with positive GBS while 10 cases with hypertension in pregnancy were identified with negative GBS group.

Positive GBS was seen more in the lower-age groups; 21.8% in 15-24y, 14% in 25-34 and 6.7% in ≥35 age-group (table 2). Out of 331 cases, majority (78%) were from Yazd province and the remainder from other provinces of Iran.

Newborns' outcome

Out of the newborns from mothers colonized with GBS, no sepsis case was reported. There was only one case of anomaly in the negative GBS group. Of all the cases with positive GBS, 8 infants were hospitalized in NICU but there was no any death or IUFD† reported. Among negative GBS group there was only 4 cases of first-minute and one case of 5-minute apgar score lower than 7, no such case was identified with positive GBS group.

In terms of mean of gestational age, head circumference, hospitalization length of time ,birth weight and height no significant difference was found between the two groups.

IUFD†:Intra Uterine Fetal Death

Table 1. Frequency distribution of positive and negative GBS colonization in pregnant women

GBS test result	Number	Percent
Positive GBS colonization	57	17.2
Negative GBS colonization	274	82.8
Total	331	100

Discussion

At the later stages of pregnancy, GBS colonization can contribute to serious infections in both mothers and infants (6). Colonized GBS newborns from mothers as carriers of GBS can lead to early onset infection, pneumonia and prematurity thereby increasing the likelihood of their morbidity and mortality. The results of present study revealed that out of 331 pregnant women, 57 cases (17.2%) were GBS positive (table 1). In general GBS colonization prevalence among pregnant women has been reported between 4.7% to 60% (7, 8).

The prevalence of GBS colonization in our study concur to what reported from Brazil (17.9%) and USA (18.7%) (9,10) but higher when compared

Table 2. Frequency distribution of the positive and negative GBS colonization in different age-groups

Maternal age-group (year)	Test results		Total (%)
	+ GBS colonization (%)	- GBS colonization (%)	
15-24	29 (21.8)	104(78.2)	133(100)
25-34	25(14)	153(86)	178(100)
≥35	1(6.7)	14(93.3)	15(100)
Total	55(16.9)	271(83.1)	326(100)

P=0.036

Table 3. Outcome of the newborns of mothers with positive or negative GBS colonization

Newborn's Outcome	GBS test result	Number	Mean	SD	P-value
Gestational age (W)	Negative	274	39	1.41	0.784
	Positive	57	39.08	1.17	
Head circumference (cm)	Negative	274	34.19	3.51	0.660
	Positive	57	34.56	1.06	
Birth Weight (gr.)	Negative	274	3159.70	383.21	0.913
	Positive	57	3165.63	328.02	
height at birth (cm)	Negative	274	50.04	5.40	0.537
	Positive	57	50.49	2.22	
Hospitalization length of time (Day)	Negative	57	1.07	0.57	0.972
	Positive	274	1.07	0.26	

with other studies conducted in Kuwait (16.4%), Iran (9.1%,13.8%,14%), Korea (8.3%) and India (4.77%) (6,21,8,12,11,13). The prevalence, however is lower in comparison with the studies performed in Saudi Arabia (27%), Tanzania (23%), Slovakia (21%), Zimbabwe (60% and 46% in rural and urban resident mothers respectively), Swiss (21%) and Newzealand (22%) (14, 15, 16, 7, 17, 18).

Agricola et al found that the prevalence of GBS colonization in pregnant women and their infants were about 23% and 8.9% respectively in a hospital in Darussalam, Tanzania. They detected a higher rate of GBS positive colonization in vagina than in rectum (12.3% vs 5%). Also delivery time were significantly longer in colonized women (more than 12h) compared with non colonized cases. In addition, they could show that there was no significant difference, between GBS colonization and the risk for prolonged rupture of membrane (PROM), fever at birth, low birth weight and AIDS infection (15).

Our study indicated higher prevalent rate of GBS colonization in lower age-groups (22.7% in 15-24y) than in higher ones (5.9% in >35y) (table 2), It may be due to higher rate of sexual relationships among the younger groups. In a study conducted in Newzeland, Grimwood et al identified a 22% prevalence of GBS colonization in pregnant women and the younger age was the highest risk factors for colonization (18). Also Kim et al could show a 14% prevalence of colonization in Korea in pregnant women younger than 25years old and a reduction of this to 9% in > 35y age group. (21)

There are, however other studies in which no relation has been found between pregnant mothers' age and GBS colonization. For example in one study in Zimbabwe, Mavenyengwa et al examined 780 pregnant women at gestational age of 20 and 26 weeks and at delivery to detect GBS colonization. GBS colonization came true with 60% of rural and 46% of the urban mothers. Colonization rate at weeks 20, 26 and at delivery was 47%, 24.2%, and 21% respectively. They concluded that colonized GBS rate is significantly higher in rural pregnant women than in the urban ones. They found no significant relationship between GBS colonization and socioeconomic, demographics and pregnancy factors. Moreover early-onset colonization of GBS had a less positive predictive value for GBS colonization at delivery(7). Although the relationship between pregnant mother's age and GBS colonization is different, it seems that in our area assessing young pregnant women should be at a higher preference.

Prevalence of GBS in pregnant mothers were 17.9% in a study conducted by Zusman et al in two hospitals of Brazil. Researchers did not find a relationship between GBS colonization and hospital type, age, race, marital status, mother's level of literacy, pregnancy frequency and a history of consumption of alcohol or smoking cigarette. (9)

Conducting a research on the Indian pregnant women by Dechen et al, GBS colonization was detected 4.77% associated mostly with Candidia (36%), S. aerous (8%), and Enterococci (8%). Moreover, no statistical relationship was found between GBS colonization and age-groups as well as mothers' gravid. For mothers being at the gestational age of less than 36 weeks, 6.93% were GBS positive and the relation was significant. They concluded that GBS colonization in pregnant women has a significant relationship with age, gestational age, early rupture of membrane and preterm delivery (8). In our study there was no any case of sepsis in infants but there was only one anomaly case among the GBS-negative group. Moreover, of all the GBS-positive group, only 8 cases were hospitalized in NICU and no case of infant death or IUFD was reported. When the GBS-negative group was analysed, 4 cases had first-minute apgar score and one case of 5-minute apgar score lower than 7 and this was not found in the GBS-positive group. There was no statistical correlation between two groups in terms of mean of gestational age, head circumference, weight and height at birth and hospitalization length of time.

In Al-Sweih's et al survey, none of the infants studied were affected with sepsis (11). Also in Jahromi's et al research, out of 1197 pregnant women tested for GBS, 9.1% of the cases suffered from recto vaginal colonization of GBS (group1). Rate of bacteria transmission from mothers to newborns was 60% (66 cases) and only one infant was affected with sepsis. In their study, although no significant preterm delivery occurred in 36.3% of the GBS positive mothers (group1) but 14.3% in GBS negative mothers (group2). Also the gestational age of the newborns in the first group was significantly lower than those in the second group; this was not consonant with that of our study. Their study revealed that PROM was 6.3% in the first group whereas this was 0.5% in the second group. (p=0.001) No difference was identified between the two groups in terms of maternal complications (12).

In three teaching hospitals of Shiraz (Iran), Hassan-Zadeh et al took vaginal and rectal specimens of 310 women who had vaginal delivery and followed up their infants up to 3 months. Of all the cases studied, 13.8% were colonized with GBS. In terms of PROM and preterm delivery no significant difference was detected between the GBS positive and negative groups. One infant was affected by meningitis and CFS culture was found to be GBS positive (13).

In a study conducted by Biringer et al in Slovakia, 767 pregnant mothers in a teaching hospital were tested for GBS, out of whom 166 cases were GBS-positive. Researchers demonstrated that when maternal GBS condition is not identified, applying prophylactic antibiotic is beneficial, this is true specially in decreasing preterm delivery (16).

In regard to the results of our study it seems that there is not cost benefit to assess asymptomatic newborns of GBS colonized mothers.

Giti et al was found no significant relation between bacterial vaginosis and preterm labor, only infection with E.coli was significantly higher in the pre-term group in comparison with term pregnancies. (19)

In a research on 125 pregnant women being at the gestational age of 35-37 weeks, Bakhtiari et al took anal and vaginal specimens of their subjects and tested them via standard culture on Todd Hewith Broth and blood agar along with determining cfb gene via PCR technique. They detected colonized GBS in 10 subjects (8%) and 12 subjects (9.6%) via standard culture method and PCR respectively. The time needed for obtaining PCR results is about 2 hours whereas using culture method is 36 hours. The researchers found that GBS can be detected rapidly and reliably by a PCR assay using combined vaginal and anal secretions from pregnant women at the time of delivery. Also they found that the rate of incidence of GBS is high in Iranian pregnant women and recommended screening of pregnant women for detecting of GBS emphatically (20).

In our study no significant correlation found between variables such as the province the patients lived in, maternal hypertension and type of delivery. In Nazer's et al study the relationship between colonized GBS and a history of maternal hypertension was not significant as well (6).

Conclusion

The results of our study showed high GBS colonization in pregnant women (17.2%). Although there was not worsening of early neonatal outcome such as early onset sepsis, pneumonia and NICU admission, routine screening of GBS is recommended in all pregnant women.

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Effect of multi-modal approach on obesity management at polyclinic: An interventional clinical trial

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Abstract

Aim: The aim of this study was to investigate the effect of reduced calorie diet restricted for only three times meal per day with combination of provision of exercise facility in clinics, providing counseling on healthy lifestyle and behavioral changes, and maintenance counseling.

Methods: It was non-randomized single group pre- and post-interventional clinical trial, conducted in healthy lifestyle center of Ataturk University Hospital, in Erzurum, Turkey with 48 randomly selected obese patients with a minimum body Mass Index (BMI) index of 30 kg/m^2, who admitted to our obesity polyclinic between January 2011 and May 2012 were included in the study. A multi-intervention treatment plan, including changing physical activity, eating habits, decreasing daily caloric intake and daily meal number, provision of exercise facility in clinic, providing counseling on healthy lifestyle and behavioral changes was used. Paired samples t test and Pearson correlation analysis were performed.

Results: The results depicted that after the intervention mean body weight decreased from 92.19 ± 14.80 to 84.7±13.3 kg ($p<0.001$) and mean BMI decreased from 37.6 ± 5.7 to 34.6 ± 5.4 kg/m^2 ($p<0.001$). Pearson correlation analysis showed a significant positive correlation between duration of adherence to the program, and weight and BMI differences ($r=0.677$, $p<0.001$ and $r=0.692$, $p<0.001$).

Conclusions: Long-term multi-intervention treatment is effective in obesity management.

Key words: Obesity, clinical exercise therapy, reduced daily caloric intake, reduced daily meal numbers.

Introduction

Obesity is a leading preventable cause of death worldwide and accepted as one of the most serious public health problems of the 21st century [1]. World Health Organization (WHO) defines obesity as a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems [2-4].

The risk of high blood pressure, coronary heart disease, type 2 diabetes mellitus (DMT2), stroke, osteoarthritis, high cholesterol, asthma, arthritis, obstructive sleep apnea and poor health status increases progressively with the increase of BMI as do the risks of cancers of the breast, colon, prostate, endometrium, kidney, gall bladder and some other organs [4-6]. The risk of death from all causes, cardiovascular disease, cancer, or other diseases increases throughout the range of moderate and severe overweight for both men and women in all age groups [7].

WHO estimates that more than 1 billion people worldwide are overweight and more than 300 million are obese and the rates of overweight and obesity are increasing in all countries including Turkey. In a study conducted in Turkey in 2009, 30.9% of subjects were with normal weight, 39.6% overweight, and 29.5% obese [8].

Most commonly combination of excessive food intake, lack of physical activity, and genetic susceptibility play main role in development of obesity, although rarely endocrine disorders, medications and psychiatric illnesses may be the reasons [9].

The three major components of weight loss therapy are dietary therapy, increased physical activity, and behavior therapy[10]. Dieting and physical exercise are the main treatment for obesity. It is important to improve diet quality by reducing the con-

sumption of energy-rich foods such as those containing high fat and sugars, and by increasing the intake of dietary fibers[10]. In case of failure in treatment by dieting, anti-obesity drugs may be used to reduce appetite or inhibit fat absorption. However there are controversies, body acupuncture and auricular acupuncture can be combined for obesity management [11-13]. Bariatric surgery or intragastric balloon may be an alternative for patients with a BMI of more than 35 kg/m^2 and obesity-related complications. Before considering surgery patient should have a strong attempt to achieve weight loss through conservative means, including diet exercise, and behavioral modifications[14].

The three major components of weight loss therapy are dietary therapy, increased physical activity, and behavior therapy. Dieting and physical exercise are the main treatment for obesity. It is important to improve diet quality by reducing the consumption of energy-rich foods such as those containing high fat and sugars, and by increasing the intake of dietary fibers. In case of failure in treatment by dieting, anti-obesity drugs may be used to reduce appetite or inhibit fat absorption. However there are controversies, body acupuncture and auricular acupuncture can be combined for obesity management [11-13]. Bariatric surgery or intragastric balloon may be an alternative for patients with a BMI of more than 35 kg/m^2 and obesity-related complications. Before considering surgery, a patient should have a strong attempt to achieve weight loss through conservative means, including diet, exercise and behavioral modifications.

There are a thousands of papers published showed the effect of different modalities worldwide however in Turkey so far difficult to find any evidence to show a multi-modal interventional study. Thus we sought to aimed for investigating the effect of multi-modal approach included reduced calorie diet restricted for only three times meal per day with combination of provision of exercise facility in clinic, providing counseling on healthy lifestyle and behavioral changes, and maintenance counseling which includes continued contact with the health care practitioner for education, support, and medical monitoring.

Materials and methods

Setting and sampling

This study was conducted in healthy lifestyle center of Ataturk University Hospital, in Erzurum, Turkey. Seventy randomly selected obese patients with at least BMI index of 30 kg/m^2, who registered to our obesity polyclinic between January 2011 and May, 2012 were included in the study. In the first interview all the details of the projects were explained to all the patients of healthy lifestyle center and took an informed consent. Three patients with a TSH level out of the normal range (0.27-4.2 uIU/mL) and 19 patients who continued to exercise programs less than 4 week excluded from the study. However remaining 48 patients were continued to participate in the study. An approval has been taken from research and ethics committee from the university. Before going to start an intervention, a briefing session was conducted and explained all procedure to the participants.

Multi-modal interventional strategy

Physical activity

At least 3 day per week walking, lasting 60 minutes on treadmill in healthy lifestyle center. The walking speed was increased 500 meters per hour per week as the patients' stamina increased. Patients were told to be active for one hour on the other days when they didn't come to exercise and keep a food and activity diary. We reviewed their activity diary weekly with the patients.

Eating habits

A list of calories of the portions of cooked meals and uncooked foods were given to all the patients and they asked not to prefer foods with high calorie and high glycemic index as high insulin levels can encourage weight gain [15] and decrease their daily consumption of carbohydrates and fat by pouring back ¼ of their portion they have put in their plates at every meal. The aim of these precautions was to create a caloric deficit of 500 to 1000 kcal per day from the current level. We recommended a diet containing 1000 to 1200 kcal per day for all patients. Patients were also instructed to drink 2.5 liters water per day and not to eat any things before elapsing 5 hours from a meal,

not eat in a hurry and spend at least 20 minutes for every meal to get pleasure and satisfaction and spend enough time for chewing. In order to get use to chew their morsels long enough, at the first 2 weeks patients asked to chew their every morsels at least 20 times. Patients' weekly progress being made, food and activity diary were reviewed regularly on weekly bases.

Patient education

In order to make the patients aware of the risk factors of obesity and keep their motivation high enough, every week, 30 minutes education were given and explained in details that the major comorbidities of obesity are; coronary artery disease, other atherosclerotic diseases, hypertension, stroke, obstructive sleep apnea, asthma, glucose intolerance, insulin resistance, dyslipidemias, type 2 diabetes mellitus, polycystic ovary syndrome, osteoarthritis, back pain, gallstones, stress incontinence, menstrual irregularities, non-alcoholic hepatitis, reflux esophagitis, increased risk of breast and other cancers and increased risk of infections.

Statistical analysis

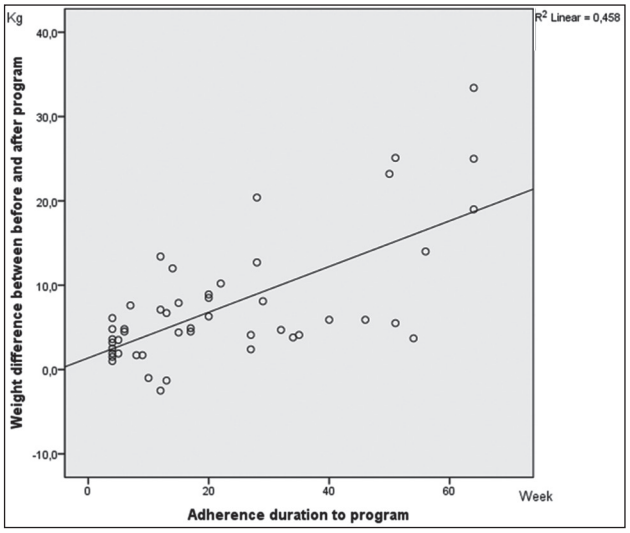
The data was analyzed using Windows SPSS version 18.0. As part of descriptive statistics, mean ± standard deviations were given where appropriate. Associations were assessed using paired samples t test and Pearson correlation analysis. Statistical significance level was set at $p<0.05$.

Results

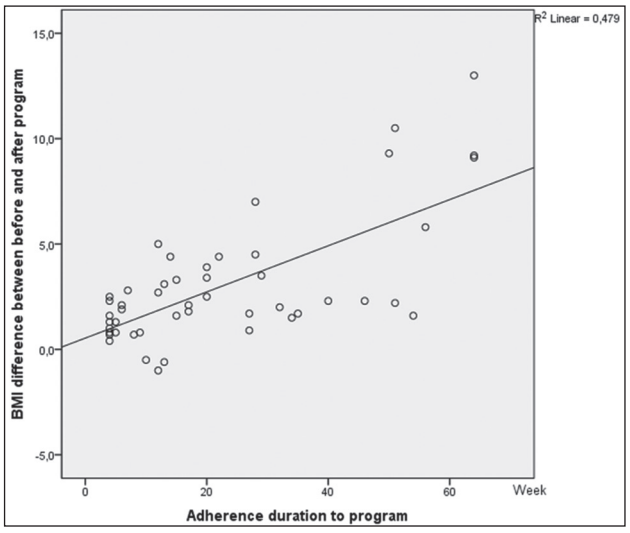
All the patients were female with mean age of 40.30 ± 10.08 years (16 - 62). Mean weight of the patients was 92,19 ± 14.80 kg (65.5 - 133), mean height was 156.5 ± 5.8 cm (144 - 170), mean waist circumference was 103.6 ± 11.52 cm (83 - 130) and, mean BMI was 37.6 ± 5.8 kg/m^2 (30.1-57.9). Average duration of participation in the program was 22.5 ±18.8 weeks (4 - 64) as shown in table 1.

After the treatment mean body weight decreased from 92.19 ± 14.80 to 84.7±13.3 kg ($t=6.95$, $p<0.001$), hence mean difference was around 7.5 kg, so reduction about 8.12%. Throughout the program, even though 45 patients lost weight (maximum 33.4 kg), 3 of the patients got some weight (maximum 2.5 kg). Throughout the program mean weight loss per patient was 7.53 kg. After the program mean BMI decreased from 37.6 ± 5.7 to 34.6 ± 5.4 kg/m^2 ($t=7.06$, $p<0.001$).

Pearson correlation analysis showed a significant positive correlation between duration of adherence to the program, and weight and BMI differences ($r=0.677$, $p<0.001$, Graph 1a and $r=0.692$, $p<0.001$, Graph 1b).



a)



b)

Graphics 1. a) Correlation between adherence duration to the program and weight difference. b) Correlation between adherence duration to the program and BMI difference.

Discussion

Our study emphasized the importance of long-term, multi-intervention treatment modality for obesity management. On the other hand diet control is thought to be the cornerstone of obesity management, however studies showed that one third to two thirds of dieters regain more weight than they lost on their diets [16]. Nevertheless it is obvious that in order to successfully lose weight, caloric intake should be reduced.

In our study in contrast to classical dietitians' recommendation, we reduced daily caloric intake as well as daily meal numbers. Since the 1960s, it's believed that there is an inverse relationship between frequency of eating and body weight, suggesting that a ''nibbling'' pattern could help to prevent obesity. This approach has later been put into question by the recognition of a high level of dietary underreporting in overweight individuals. In addition, no difference in total daily energy expenditure has been documented as a function of daily meal number [17] We believe that weight loss is not facilitated by high meal frequency, since it is proved that snacking in obese subjects is associated with higher energy and fat intake [17]. The physical activity plays an important role in reduction of weight [18, 19]. Our study is a strong witnessed to this evidence as in addition to diet control we also made a structured plan for exercise that helps a lot and showed a dramatic change in mean weight consistent with other study [20] and also overall significant reduction in BMI [21]. The second thing which is important is the education of patients to aware them about the results of obesity [4-6]. We included this part as a third strategy in our multi-modal intervention.

In a study it is found that combination of exercise under supervision, dietician consultation, and providing exercise opportunities would be an effective approach in weight loss and will be beneficial in the management of obese women [22]; which is consistent to our approach.

It is important to ensure that patients are ready and enthusiastic about attempting weight loss as their cooperation is essential. It is shown that obese patients with high motivation for weight loss would attend the program more regularly, lose more weight during the program [23]. We made a supportive interpersonal environment with our patients and maintained counseling throughout the program.

Expert panels and governmental guidelines now recommend that obese persons seek modest reductions in body weight rather than striving for "ideal" weights [21]. Generally it is assumed that a program offering a 10% reduction from initial weight and maintaining it for one year is very successful. Weight loss as little as 5% should be regarded as reasonable, as there is considerable improvement in associated risk factors. Initially our goal was to make an overall 10% reduction in mean body weight, but average weight loss per capita was 8.12 % reduction of body weight. This result reinforced other studies [19] and categorize our program as quite successful one.

These kinds of programs need a continuous motivation[24] for compliance and adherence to program and our study showed that there was a correlation between adherence to the program and weight deference before and after the program which is consistent to literature [20]. Thus it is also indicated that our program was successful to maintain the motivation and adherence.

Conclusion

By keeping the patients' motivation high, making it easy to adhere to weight loss program, providing clinical exercise facility, giving education and decreasing daily energy consumption by reducing daily meal numbers and portion size is effective in obesity treatment.

Disclosure

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Comparative analysis of the destructive stress obtained by different types of splints

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Abstract

Aim: Aim of this study was to choose a combination of reinforcement and semi-flow composite material with the highest strength parameters.

Material: The study material consisted of eight types of samples made of different types of reinforcement (fiberglass and metal). The matrix material was semi-flow composite material Flow-It.

Method: Samples in the beam form with dimensions of 25x4x1.7 mm were examined with three-point bending test on a machine INSTRON type TM-SM. Each type of sample was examined three times. In the examination destructive stress and the relative deformation results were estimated. The arithmetic average of measurements was calculated.

Results: In the study, the highest value of destructive stress (115.5 MPa) and of a relative deformation (18.5%) were obtained for a sample that combines reinforcement Fibre-Kor with a parallel set of fibers and warp Flow-It. The lowest value of both parameters destructive stress (77.2 MPa) and relative deformation (10.6%) were at a combination of reinforcement Fibre Splint Multi Layer and Flow-It matrix material.

Conclusions: Strength tests of reinforcement – warp composition let you limit the number of complications such as rupture or destruction of the splint.

Weave with a parallel arrangement is advantageous to strengthen of the splint.

Good wetting of the fibers by the matrix material and an appropriate adhesion permit to obtain good strength parameters.

Key words: Stabilization of teeth, destructive stress, relative deformation.

Introduction

In recent years, the factors that increase host susceptibility to periodontal disease were determined. It can be divided into two groups:

- determinants such as gender, age, social status, genotype - the control and predisposing factor for periodontal disease,
- appropriate risk factors - factors that modify the host response, making it susceptible to the occurrence of periodontal disease. To these we include: diabetes, osteoporosis, smoking, disease which are manifesting as immune deficiencies: congenital (Down's syndrome) and acquired (AIDS).

The primary symptoms of periodontal disease can include: inflammation, destruction of periodontal and bone tissue. Inflammation of the gums is caused by plaque and its products, which over time reach the deeper periodontal tissues which keep the tooth in the alveolus. The escalation of this process leads to the migration of epithelial attachment and destruction of the periodontal fibers also their attachment to the bone and cement. In a further stage they lead to the alveolar bone resorption of the jaws and then teeth become mobile. The method that allows avoiding or delaying the loss of teeth is mobile teeth stabilization. It helps a weakened periodontal function by creating a dental block, which will be more effectively carrying the force of chewing [1].

Frequently the choice from a wide range of materials takes place without knowing the mechanical properties. The decisive role is taking aesthetic parameters, the way of application and economic aspects. The object of these studies were selected connections of matrix and reinforcement (elements which build the splint) used for mobile teeth stabilization. During the static 3-point bending tests there were examined parameters such as destructive stress and relative deformation of the materials. The results of comparative studies give possibility to optimize the selection of the composite matrix and reinforcement for individual clinical use. Those examinations should be made in order to ensure

less complication in the form of cracks and damage of the stabilized mobile teeth [2].

Materials and methods

The study material consisted of 8 types of reinforcement made of glass fiber (Fiber Splint, Fiber Splint Multi-Layer, Fibre - Kor twisted and parallel fibers, Splint-It Linke and reticular arrangement) and metal (Retainer, Splint Lock) which were connected with Flow-It warp. They formed a sample in analogous way to the splints used in the stabilization of mobile teeth (Table 1).

Table 1. Summary of samples used in examination

Sample No	Material	
	Reinforcement (splint)	Warp material
1	Fiber – Splint	Flow – It
2	Fibre – Kor (twisted allignment)	Flow – It
3	Fibre – Kor (paralel alignment)	Flow – It
4	Splint – It (check alignment)	Flow – It
5	Splint – It Linke	Flow – It
6	Splint – Lock	Flow – It
7	Fiber Splint Multi-Layer	Flow – It
8	Retainer	Flow – It

The samples were made in the shape of beams with dimensions of 25x4x1.7mm. They have been crafted in a specially prepared plexiglass module according to the materials manufacturer recommendations. All those procedures were carried out analogous to the clinical application conditions. Samples were cured with the Heraeus-Kulzer lamp. Each sample was treated for 60 seconds.

To analyze the mechanical parameters of samples of materials used to stabilize mobile teeth they were tested in three-point bending examination, which most closely reflects the loads occurring in the mouth during mastication. This study is normalized using PN-79/C-89027 and ISO4049 standards [3,4].

This examination allows to determine, inter alia, the breaking tension and the value of relative deformation. Evaluation of these parameters allows to choose the optimal selection of reinforcement and matrix components, which will be the most durable in the clinical use. These studies reflect an extreme situation, leading to the destruction of the composite, and therefore should be regarded as comparative materials research [5].

The three-point bending examination was performed on machine Instron type TM-SM in the Department of Materials Science Technical University of Bialystok (Figure 1).

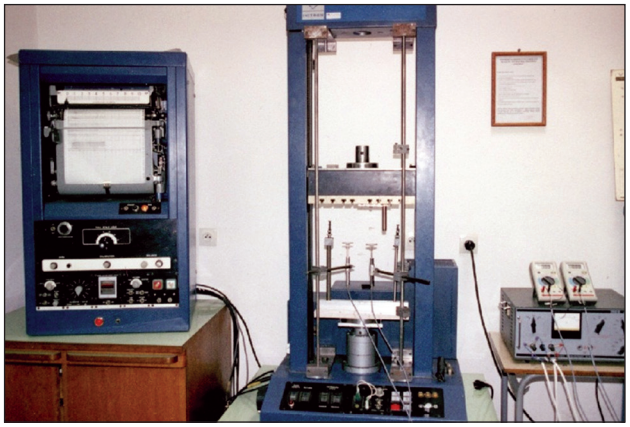

Figure 1. Machine INSTRON type TM-SM

This unit during the study used the strain gauge measuring head with the burden DRM: 10, 20, 50, 100, 200, 500 kilograms and worked with the following parameters: speed of the machine traverses 1mm/min, speed recorder 20cm/min, measurement range extensions - max 112cm. This study is based on giving the point load at the prepared sample, which was supported at the ends. The distance between the points of support is strictly defined according to standardization. In this case, the applied load is placed symmetrically with respect to the supports. The load has been given uniformly, until the destruction of the sample [6, 7, 8]. Performing of the test using INSTRON machine type TM - SM required a special sample holder (Figure 2).

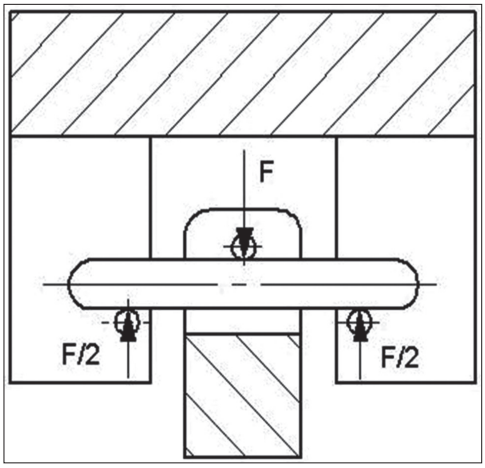

Figure 2. Handle with the sample used in the three-point bending test

All the samples were placed in the holder and tested three times. On the basis of the force which was exerted and the deformation of the sample the values such as relative deformation and destructive stress were estimated. The arithmetical average was taken from the results.

Results

The highest value of the destructive stress 115.5 MPa obtained a sample built from Fibre-Kor - fiber glass reinforcement with parallel arrangement of fibers and the matrix Flow - It. Equally high values of this parameter obtained metal splint - Splint Lock and Flow - It. The lowest value 77.2 MPa achieved connection of reinforcement Fibre Splint Multi Layer and warp Flow - It. Equally low value 80.9 MPa of destructive stress obtained a sample which is a combination of reinforcement Fibre-Kor with twisted arrangement of fibers and matrix Flow-It (Table 2). The highest value of relative deformation – 18.5% received a combination of reinforcement Fibre-Kor with parallel arrangement of fibers with the matrix Flow-It. Relatively high value of 13.3% obtained a splint made of Fibre-Kor with twisted arrangement and Flow-It warp. The lowest value of this parameter (10.6%) obtained of the sample made of Fibre Splint Multi Layer and material Flow-It (Table 2).

Discussion

In the case of splints made of the same type of fiber, but with different spatial layout (Fibre-Kor with parallel and twisted alignment and Fiber Splint arranged in single and Multi-Layer system), it is clear that the simpler spatial arrangement allows for a higher mechanical properties and also allows the larger relative deformation. It helps to achieve greater susceptibility of the splint. It can probably be explained by a good wetting between the fibers with the material that can easily flow into it. Probably this material has also good adhesion to fibers. The Fibre-Kor reinforcement with parallel arrangement can also take higher loads and has proved to be almost twice more flexible than metal reinforcement splints. Due to the fact that all samples were compared with the same type of matrix it can be assumed that the main influence on its parameters had reinforcement which was used and its spatial arrangement.

Conclusion

Strength tests of reinforcement - warp let us limit the number of complications such as rupture or destruction of the splint.

Parallel arrangement is advantageous to mechanical properties of the rail.

Good wetting of the fibers by the matrix material and an appropriate adhesion allow to obtain good strength parameters.

Table 2. Results of three-point bending tests

Sample No	Material		Relative deformation e, %	Destruction stress s_z, MPa
	Reinforcement (splint)	Warp material		
1	Fiber - Splint	Flow – It	13	91.3
2	Fibre – Kor (twisted fibres)	Flow – It	13.3	80.9
3	Fibre – Kor (paralel fibres)	Flow – It	18.5	115.5
4	Splint – It (reticular alignment)	Flow – It	11.9	94.3
5	Splint – It Linke	Flow – It	11.7	89.5
6	Splint – Lock	Flow – It	11.6	102.7
7	Fiber Splint Multi-Layer	Flow – It	10.6	77.2
8	Retainer	Flow – It	10.9	91.9

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An electronic learning tool to improve language related communication skills in healthcare settings

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Abstract

An electronic learning tool (ELT) including common words and phrases used in healthcare staff -patient communication and some sample video clips in seven different European languages was developed. We aimed to test effectiveness of the ELT in a Turkish study group representing different healthcare branches and community. First, level of verbal English skills of the participants was determined. Following two-month self-studying period, the participants were re-tested. Their satisfaction with the process was determined one month later than the post-test. A significant progression was observed between pre and post-tests. Satisfaction with the process was high. Satisfaction and test scores correlated significantly. The ELT with its aim-specific content for healthcare services seems effective on improving verbal communication skills at least in English.

Key words: Healthcare, patient, healthcare staff, communication, foreign language.

Introduction

Health care, especially patient-staff relation is totally based on a sound communication without which correct diagnosis, effective examination and treatment can never be achieved. Some communication barriers between health staff and patients may appear from lack of foreign language capabilities. Misunderstandings due to these communication barriers not only complicate the individual care and medical outcomes, but also lead to financial and social problems like workforce loss via medical complications (Martin, 2006).

The quality of communication can be enhanced with specific training activities like improving language skills of healthcare staff (Bischoff et al., 2003). However, health professionals hardly find enough time to learn a foreign language because of their excessive workload. An easy to access and usable multimedia software including text and audiovisual facilities together would be highly appreciated by healthcare staff and patients who need to communicate in a foreign language.

In such a situation, computer assisted language learning (CALL) might be the preferred strategy to increase their foreign language skills. CALL is an approach simply defined by Levy as the search for and study of applications of the computer in language teaching and learning (Levy, 1997). CALL is a general label and covers wide range of ICT applications and approaches to teaching and learning foreign languages (Levy & Hubbard).

The current philosophy of CALL puts a strong emphasis on student-centred materials that allow learners to work on their own. CALL materials are generally designed regarding principles of language pedagogy and methodology derived from different learning theories (e.g. behaviourist, cognitive, constructivist) and second language learning theories.

The aim of this study was to test effectiveness of an electronic learning tool prepared in different European languages by the authors to help people learn basic statements required for an effective patient-staff communication in health care services.

Material and methods

An electronic learning tool (ELT) including aim specific words and phrases in seven different European languages was developed by a multinational project. Effectiveness of the ELT was tested in a prospective study via determining contribution of the ELT to improving English verbal skills of a sample of Turkish target population.

A. Establishment of the ELT

1. Contributors

A multinational project was conducted to develop the ELT under the frame of *"European Commission – Education &Training - lifelong learning programme"* and carried out between December 2007 and November 2009. Six institutions from five different European countries including universities, hospitals, and companies took part in the project as partners. Each partner established an internal feedback group composed of representatives of target population to have their recommendations on materials produced in each stage of the project. Additionally, an institution experienced in teaching foreign languages externally evaluated all project process.

2. Preparation of the content-Workflow

a. Determining needs: Each partner, in their countries, determined most commonly used words, phrases and terms in communication between healthcare staff and patients via semi-structured questionnaires, and interview or observation forms. Data was gathered from physicians, nurses, dentists, pharmacists, medical secretaries, physiotherapists, dieticians, laboratory workers, hospital cashiers and workers, drivers, travel agencies and patients. All obtained words and phrases were translated into English and combined in a single list. This list was placed in the official website of the project to take recommendations of visitors.

b. Translation: The list of commonly used words and phrases in English was translated into other target languages which are Turkish, German, Bulgarian, Czech, Latvian and Russian by professional translators in partners' countries. Feedback groups and professionals provided feed-back on translated lists. After some revisions, final form of the list in 7 target languages was shaped. The list was divided into two main sections as "vocabulary" and "phrases". The "phrases" section was also divided into 11 subsections regarding occupation groups from whom the data was gathered.

Additionally, ten short dialogs in English were created by the partners to be used in video recordings. The dialogs were about communication in some basic physical examination or medical intervention procedures as well as some registration formalities. These scripts were also translated into all target languages and recommendations of feedback groups were received.

c. Audio and video recordings: Each word and phrase in the list was separately dubbed by the partners in a quiet, echo free place. The recorded audio files were stored in "wav" format. Video clips were shot by the partners using previously determined scripts and stored in "MPEG" format.

d. Preparation of the software: An easy-to-use interface (JMF 2.1.1e Software) was selected to create project product. Final text forms of vocabulary and phrase list, audio and video files were embedded into the software. Subtitles in seven target languages were added into the video files. Whole content (words phrases and subtitles) was designed in an order that made matching possible between target languages. Prepared software was presented to the feedback groups and individuals were asked to perform some tasks using it. After having recommendations of feedback groups, final form of the software was shaped and saved onto a DVD. Original DVD was multiplied and delivered to target institutions such as hospitals and primary healthcare centres, travel agencies or hotels in 5 different countries. The software is also available as "online Wiki form" in "project results" section of official website of the project (http://www.elancom.eu).

B. Testing the effectiveness of ELT

Verbal communication skills of a Turkish target population in English were studied to test contribution of the ELT to language related communication skills.

1. Study group

One hundred and one voluntary Turkish people representing different branches of health care and community participated in the study. A special attention was paid to studying with individuals who frequently need to communicate with others in English in their work settings, but suffer from communication problems because of their limited English proficiencies.

2. Evaluation, training and re-evaluation

An English native speaker, who teaches English to Turkish students, tested the verbal skills of the participants in English on the basis of "words" and

"phrases" sections of the ELT relevant to occupation of each participant. For example while testing verbal skills of a nurse in English; the assessor limited himself with the content of nursing related words and phrases in the ELT. After determining their verbal skills, we delivered a DVD including the content of the ELT to each partner and trained the participants in small groups to teach how to use and self-study the ELT. In order to help the participants, the phrases allocated to health care professionals and patients were separated using different colours in text form of the ELT. Ten people, whose computer using skills seemed insufficient to use the ELT, received extra training on general principles of computer use. Training took three weeks. Following the training, the participants self-studied the relevant part of the ELT throughout two months between the first of May and the first of July 2010. The method of learning was memorizing the relevant words and phrases at first. Then the participants created dialogs from the content of the ELT and studied these dialogs with the people in their work settings or friends as pairs. Some of them also found an opportunity to use gained knowledge in communication with foreigners. After self-studying period, the same assessor re-tested the participants' language skills. Performances in initial and final oral tests were scored over 100 points.

3. Satisfaction of the participants with the ELT

One month after the final test, a satisfaction questionnaire prepared by the authors was delivered to the participants to determine their satisfaction with the ELT and learning process. The satisfaction questionnaire included 15 statements and the participants were asked to give a score for each statement on a five item Likert-type scale between 1 (absolutely not agree) and 5 (absolutely agree). An empty place was left for the participants at the bottom of the questionnaire to have their views on usability of the tool, self-studying period and real life experiences.

C. Data analyses

The difference between initial and final assessment scores was investigated using Wilcoxon signed ranks test and paired samples t test. The correlation between satisfaction levels and each of final test score and improvement in test scores was analyzed using Pearson correlation analysis. Factors predicting final test success and satisfaction with the process were investigated using multiple regression analysis. The independent factors analyzed to predict final exam success were profession, educational background, gender, initial test score, computer using skills and satisfaction with the ELT. In order to determine predicting factors for satisfaction of the study group, we analyzed profession, educational background, gender, initial and final test scores, computer using skills and degree of improvement between two test scores as independent factors

Results

Content of the tool

The number of the words and phrases in "vocabulary" and "phrases" sections of the ELT was 1150 and 1232 respectively. Distribution of the phrases according to professions is presented in Table 1. Some phrases, especially those related to the "first meeting with the patient" were repeated in all profession groups. Consequently 1446 phrases took place in final form of the ELT. Of these phrases, 518 were related to patients and, again, some of them were repeated in different sections (Table 1).

Study group

Mean age of the participants was 34.7±6.5 years. Male/female ratio was 3/2 and all of the participants graduated from high school (22.8%) or university (77.2%). Information about professions and computer using skills of the participants are presented in table 2.

Effectiveness of the ELT

Every participant improved oral test score following the training. Mean scores attained in pre and post training oral tests were 51.9±11.8 and 80.4±9.2 respectively. The difference between them was statistically significant (Table 3).

Table 1. Distribution of the phrases according to health-care professions

Profession	Number of related phrases
Physician	**545**
- First meeting	*19*
- Medical history	*215*
- Physical examination	*96*
- Information and suggestions to the patient, medical tests and prescribing	*147*
- Patient questions and wishes	*68*
Nurse	**221**
- First meeting	*21*
- Information, questions, suggestions and warnings	*170*
- Patient questions and wishes	*30*
Dentist	**167**
- First Meeting and examination	*69*
- Information	*36*
- Suggestions and warnings	*22*
- Patient questions and wishes	*40*
Pharmacist	**70**
Secretary	**131**
- First Meeting, registration	*46*
- Information	*49*
- Patient questions and wishes	*36*
Physiotherapist	**89**
Dietician	**49**
Laboratory worker	**37**
Cashier desk	**45**
Hospital worker	**30**
Driver	**62**
All	**1446**

Overall mean satisfaction score with the ELT was 4.14±1.03 in the study group (Table 4). There was a statistically significant correlation between post training test scores and total satisfaction scores (r=0.61, p=0.000). Satisfaction scores were found to be increasing as the difference between the scores of initial and final oral tests increase (r=0.66, p=0.000). When all independent variables were set in multiple regression analysis, initial test score (t=9.766, p=0.000), profession (t=-3.392, p=0.001) and satisfaction (t=4.484, p=0.000) were found to be predicting success in final test. Predictors of satisfaction with the process were degree of improvement between initial and final test scores (t=4.373, p=0.000) and profession (t=2.125, p=0.036).

Table 2. Characteristics of the study group

Characteristics	n*(%)*
Gender	
Male	61*(60.4)*
Female	40*(39.6)*
Educational background	
University or higher	78*(77.2)*
High school	23*(22.8)*
Profession	
Physician	11*(10.9)*
Nurse	7*(6.9)*
Dentist	6*(5.9)*
Pharmacist	8*(7.9)*
Secretary	12*(11.9)*
Physiotherapist	5*(4.9)*
Dietician	5*(4.9)*
Laboratory worker	6*(5.9)*
Hospital worker	4*(4.0)*
Driver	4*(4.0)*
Out of health care	33*(32.7)*
Computer using skills	
High	72*(71.3)*
Enough	19*(18.8)*
Low	10*(9.9)*

Some samples from views of the participants about their experiences:

"I participated in the study as representative of community. I suffer from a rheumatologic chronic disorder called ankylosing spondylitis. I believe that I achieved a mastery level in words and phrases relevant to my disease and I can use them when I need."

"I would benefit more from the learning tool if it contains a grammar teaching part. I always learn better if the knowledge is presented in a well-structured manner"

"You may add a "test yourself section" into the ELT. Using this section it would be possible for me to see my personal progress."

"I suffer from a slight form of dyslexia; I always had some problems with structured classical teaching methods. I learn better by listening and watching. I like this tool since it does not contain complex text, graphic or table samples."

"It was amazing to see that aim specific conversation in my job is possible using limited number of words and phrases. In my experiences throughout the last month, I was able to express myself effectively. I also started to catch key points from

Table 3. Initial and final verbal test scores of the study group

Profession	Initial oral test	Final oral test	Z*	p
Physician	63.5±9.5	90.3±6.8	-2.950	0.003
Nurse	49.9±10.0	74.8±4.7	-2.366	0.018
Dentist	62.5±5.9	88.5±2.9	- 2.207	0.027
Pharmacist	60.2±7.5	87.0±4.4	-2.524	0.012
Secretary	50.1±11.4	81.8±7.7	-3.063	0.002
Physiotherapist	52.4±6.4	74.4±7.1	-2.032	0.042
Dietician	48.4±4.1	80.0±5.8	-2.032	0.042
Laboratory worker	43.7±7.9	78.0±6.7	-2.201	0.028
Hospital worker	35.5±7.5	81.0±6.2	-1.841	0.066
Driver	39.0±7.7	72.5±3.0	-1.826	0.068
Out of health care	50.6±11.8	77.0±10.4	-22.603**	0.000
Overall	51.9±11.8	80.4±9.2	-34.498**	0.000

** Wilcoxon signed ranks test*
*** Paired samples t-test, t value*

Table 4. Satisfaction of the study group with the ELT

Statements	Satisfaction score Mean ± SD
My computer skills were enough to use this tool	4.39±1.23
Knowledge and skills that I gained in this period will contribute to my professional life achievements	4.04±1.11
I would like to benefit from similar electronic tools in the future	4.23±1.14
*It took too much time to learn with this tool	3.44±1.1
I learn better with this tool	3.86±1.15
This tool had positive effect on my motivation to learn	4.54±1.13
*I faced some technical difficulties while using this programme	3.70±1.10
It was enjoyable to learn via this tool	4.39±1.14
*This method does not contribute to my learning	3.86±0.97
The content was enough to meet my requirements	4.00±1.06
* It was stressful to find a computer	4.01±0.20
I used the phrases that I learned here in communication with foreigners successfully.	3.97±1.22
I expressed myself better in communication with foreigners using the phrases that I learned here	4.01±1.33
I understood foreigners better with the help of phrases that I learned here	3.29±1.13
In general, I am satisfied with this tool and process	4.14±1.03

** These statements were scored reversely regarding their negative meaning*

statements while I am listening to a foreigner. Now I see that, the most important thing is self-confidence in communicating with foreigners"

"Although I performed much better in the final oral test, I am still doubtful about the effectiveness of the tool. I accept that I can express myself using the statements that I learned here. But I still hesitate to talk to foreigners due to fear of not understanding what they say."

Discussion

We aimed to test the effectiveness of a multinational project product (ELT) which is available in seven different European languages and presenting commonly used words and phrases in healthcare staff-patient communications. For this aim, we investigated contribution of the ELT to English speaking skills of a Turkish target population composed of people with limited English proficiency. Limited English proficiency is the limited ability or inability to speak, read, wri-

te or understand the English language at a level that permits the person to interact effectively with others (Jacobs et al., 2003).

Effective communication between patient and healthcare provider is critical to the delivery of safe, high-quality care. Language barriers can impede patient–provider communication (Cohen et al., 2005). The inability to effectively communicate with a provider limits patient access, weakens trust in the quality of the medical care received and decreases the likelihood that patients will receive appropriate follow-up (Flores et al., 2002). In addition, language barriers can result, on the part of the patient, in misunderstandings, problems with informed consent, inadequate comprehension of diagnoses and treatment, dissatisfaction with care, preventable morbidity and mortality, disparities in prescriptions, test ordering and diagnostic evaluations (Flores et al., 2002). On the provider side, language barriers can inhibit a clinician's ability to elicit patient symptoms, often resulting in an increased use of diagnostic resources or invasive procedures, inappropriate treatment and diagnostic errors (Ku&Flores, 2005). Patient–provider communication is also a serious patient safety concern and a common root cause of adverse events in healthcare (Joint Commission Sentinel Event Data, 2010). An adverse event is any unintended harm to the patient by an act of commission or omission rather than by the underlying disease or condition of the patient (Aspden et al., 2004).

The need for improving communication skills in foreign languages is inevitable for both of healthcare providers and patients. Some attempts on this purpose have been made so far, for example books were published or some electronic materials or web sites were established to improve reader's or user's language skills (Hull, 2009; Glendinning&Holmström, 2004; English Language Centre, University of Bath, 2010; HospitalEnglish.com, 2010). However, previous publications or electronic/web based self learning materials generally targeted health care professionals and patient side was ignored. Our product differs from preceding works in its content including special statements both for health care professionals and patients or service takers.

Our study group showed significant improvement in their verbal skills after two-month self-studying period. Initial level, profession, and satisfaction with the learning process were found to be predicting success in final test. Initial level (i.e. first test scores) was higher in some profession groups like physicians, dentists or pharmacists than that of other professions. Consequently, their final test scores became higher too. Then, it is an expected finding to determine initial score and profession as indicators of final test success. The third predictor for success was satisfaction with learning process. We also found a strong correlation between post training test scores and satisfaction scores in correlation analysis. Therefore, satisfaction of the participants seems to be the key point on the way to success and should be discussed comprehensively.

We received no criticism about the content of the ELT from our participants and the mean score given to the relevant item in the satisfaction questionnaire was high. The reason behind high satisfaction with the content of the ELT may be the similarity between our study group, and the population from whom data was gathered to establish the content. Adults learn better when they contribute to content of educational material and see that the content is relevant to their needs in professional life or specific needs (Kurt, 2000). This adult learning principle may explain high overall satisfaction score of the study group with the process. But we think –and multiple regression analysis showed- that the most important factor affecting overall satisfaction is the improvement in verbal language skills. Computer based self-learning which offers independent and individual learning opportunities without any time and place limitation may have contributed to high satisfaction too. Profession was the second variable that predicts satisfaction. Professions represented by relatively less number of statements in the ELT content, predicted higher satisfaction. The reason behind this may be that participants from these professions showed the same progression with others giving relatively less effort.

Each individual learns in a distinctive way and adopts different learning styles. Some people learn best through hearing information, some through seeing visual images, and some through actual hands-on experience. Some people take in information through thinking through ideas and concepts and some through the feelings associated with specific examples and experiences (Knowles et al., 2005).

Differences between learning styles of our study group were expressed well in the qualitative data. Some participants stated that this learning style was ideal for them, where as, some others would prefer more structured and grammar based content. Considering different learning styles in training programs is crucial to increase learner engagement. In a study conducted among Turkish adults, learning styles were found to be related to satisfaction with learning process and academic achievement (Gurpinar et al., 2010). In response to these issues, CALL seems to be an appropriate approach since one of the main promises of it is the ability to individualise learning allowing learners to proceed at their own pace. (Warschauer, 1996).

Limitations

ELT: The limitations of the ELT may be held in frame of basic phases of CALL which were developed regarding behaviourist, cognitive and constructivist learning theories. First phase was behaviouristic CALL. Programs of this phase entailed repetitive language drills and can be referred to as "drill and practice". Second phase was communicative CALL which focused on authentic communication. The last phase is integrative CALL which allows combining reading, writing, speaking and listening skills by use of different multimedia facilities. (Warschauer, 1996). Our ELT has some behaviouristic components since it offers a learning environment that requires self learning memorizing relevant words and phrases. Although it has not focused use of language in self learning period, the first and final assessments and the content maybe associated with communicative CALL approach. It also has some integrative elements since it offers a learning environment for reading and listening skills using multimedia facilities. Defects of the ELT according to descriptions of three phases of CALL naturally reveal its limitations. For example lack of a grammar teaching component, a feedback mechanism or a "*test yourself*" section is the first limitation of the project product criticized by the participants. Existence of such sections would provide an opportunity for the users to better drill and practice opportunities for behaviouristic CALL approach. Although the content is authentic, lack of opportunities for speaking and writing in the ELT is another limitation from the points of communicative and integrative CALL approaches. An additional limitation is providing no links to language authorities to ask for language related help or support.

There is no doubt that the ELT in its current form is not a fully equipped learning media. It may only be used as a supportive material in an e-learning program. If an e-learning program is implemented using the ELT, four major themes determined by Coryell & Chlup (2007) in a nationwide survey and focus group study conducted among directors and instructors of adult English language learning programs, should be considered. These themes are (1) careful and detailed preparation, (2) individualized, student-centred instruction, (3) support, and (4) collaboration.

Study design: The most prominent limitation was sample size. Our study group composed of a few representatives of each target profession living in the same city. Therefore, our results cannot be generalized to whole target groups in Turkey. We announced the second assessment date at the beginning of the study and the participants prepared until that date. This was the second limitation. If we performed the second assessment at random dates by inviting the examinee one day prior to the assessment date, we would obtain more realistic performance scores indicating readiness-not preparedness- levels of the study group.

Conclusions

Our project to develop the ELT is the first and very early step to bring solution to language barriers between health-care providers and patients in different languages. In time, we are sure that it will be improved by other future projects both in terms of technical aspects and content. Our language learning tool with its aim-specific content for health care services seems to be effective on improving language related communication skills at least in English. We are aware that results of this study cannot be generalized to other populations and languages. However, similar studies may be planned to test the generalizability of our results. For this purpose, the ELT used in this study seems to be an appropriate instrument since it contains aim-specific words and phrases in seven different European languages.

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Tuberculin Skin Test and BCG scar in children vaccinated at birth: A study from Iran

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Abstract

Background and objectives: The rates of positive tuberculin skin test reactions and BCG scarring after BCG vaccination vary between studies and populations. Tuberculin reactivity and BCG scarring may be related to better child survival in low-income countries.

This study was performed to assess the reaction to the tuberculin skin test and the development of the BCG scar in a cohort of healthy newborns.

Methods: We performed the tuberculin test with Purified protein derivative (PPD) and the BCG scar in 794 infants 3 months after vaccination at birth in 2010 at the Taleghani hospital, Arak city, Iran

Results: Of all children 413 were female (52%). Response to the tuberculin test was negative in 39%, doubtful in 55% and positive in 6%. In 96% of cases B.C.G scar was ≥3 mm in diameter. The average size of the reaction to PPD in those with a BCG scar was greater than those without a scar in all the children in our study, (p-value < 0.001).

Conclusions: In the present study, about 96 % of infants had a successful response to BCG vaccination while 4% did not develop a scar. In some countries, repeat vaccination is universal; in others it is based on either tuberculin skin testing or the absence of a typical scar. Since the absence of development of the scar does not always signify a lack of response to the BCG, therefore it is better to do further immunological tests to detect a reaction to the vaccine before deciding to revaccinate these children.

Key words: BCG vaccine, Tuberculin Skin Test, scar.

Introduction

BCG vaccine, with more than 80 years antiquity, is still the most used vaccine around the world. (1) Approximately 100 million children receive the BCG vaccine every year; the vaccine is given routinely in most countries of the world. As reported to WHO in 1998, global vaccination coverage of infants with BCG by 12 months of age was about 85%. (2) Reports about the induction of immunity and the rate of effectiveness of this vaccine vary between 2-83 percent in different studies. (3)

Estimates of protection of BCG against pulmonary TB vary from 0 to 80%, but it is believed to confer a higher rate of protection against miliary TB and tuberculosis meningitis in childhood. (4, 5, 6). Tuberculosis skin test, (TST) reaction and BCG scar have been much debated as markers of BCG efficacy against TB but have lately been shown to have limited validity. (7) On the other hand, literature search shows that BCG-vaccinated children with a BCG scar or a TST reaction had a better survival rate than BCG-vaccinated children without a scar and no TST reaction. (8)

The tuberculosis skin test is a test used to determine if someone has developed an immune response to the bacterium that causes tuberculosis (TB). This response can occur if someone currently has TB or if they were exposed to it in the past. The tuberculin skin test is based on the fact that infection with M. tuberculosis produces a delayed-type hypersensitivity skin reaction to certain components of the bacterium. This reaction begins when T cells, which have been sensitized by prior

infection, are recruited by the immune system to the skin site where they release lymphokines. These lymphokines induce indurations through local vasodilation, edema, fibrin deposition, and recruitment of other inflammatory cells to the area. (9) Vaccination with live viruses may interfere with the TST reactions. (9)

The number of positive TST in previously BCG-vaccinated individuals has varied greatly depending on the population and the set-up of the study, and a BCG vaccination does not always result in a scar.(3) The size of the TST reaction, and to a lesser extent BCG scar, varies with the dose of viable bacilli of BCG according to a dose-response relationship.(11,12) The route of administration, subcutaneous, or intradermal, had a marked effect on the TST reaction in one study but no effect in another, (13,14). Malnutrition, viral infections (measles, varicella and influenza) or vaccines, (measles and polio), may suppress the TST reaction. (11,12,15)

BCG vaccination used for the prevention of tuberculosis may cause problems in interpreting the tuberculin skin test, which is commonly used in the diagnosis of infection. A limited number of studies have been undertaken to investigate how the passage of time after BCG vaccination affects TST results.

Since a small number of studies have examined the TST reaction and BCG-scarring in infants within the Expanded Program on Immunizations, (EPI), in Iran; therefore, this study was done to determine the TST response and the BCG scar 3 months after the routine BCG vaccination at birth, in a cohort of healthy newborns.

Materials and methods

The study included 794 children. The inclusion criteria were all healthy neonates who were born and registered at the Taleghani Hospital in Arak city in 2010 and received BCG vaccination at birth. All vaccines were supplied to the vaccinating centers through the local EPI program.

In a cohort study of children who were born in the study area, 0.5 ml of BCG vaccine was given intradermally, by injection with a 25 or 26-gauge needle, in the deltoid insertion region of the upper arm at birth. The TST was administered by a trained nurse 3 months to the volar surface of one of the forearms intradermally by injecting 0.1mL of 5 TU PPD (SSI RT232T.U.Pasteur Institute in Iran) after obtaining written consent from their mothers. The reaction was measured in millimeters of induration, (palpable, raised, hardened area or swelling), between 48 and 72 hours after the injection. The nurse did not measure erythema (redness). Finally, children were classified into three groups according to PPD reaction: positive (>=10 mm), doubtful (5-9 mm) and negative (0–4 mm). Besides, BCG scar formation was examined. BCG scar was defined as positive if it was equal or more than 3 mm. and negative if it was less than 3 mm.

Statistical methods

Mean and standard deviation were applied for quantitative data and frequency (%) for qualitative variables. T-test was employed to assess the significance of differences between group means.

Results

In this during 7 months study, of 794 infants , 413 (52%) were female and 381(48%) were male. PPD induration size was 5.3± 1.4 mm in females and 5.7±1.9 mm in males. The difference in PPD size between two sexes were significant (*4.3 ± 1.7 mm versus 4.4±1.5 mm, p-value* <0.001.). Response to tuberculin test has been negative in 309 persons (39%), doubtful in 436 persons (55%) and positive in 49 persons (6%). (Table 1)

In this study, B.C.G scar in 96% of cases was ≥3 mm in diameter and only in 4% was negative. The difference in the size of scar between the two sexes was not significant (*4.4± 1.5 mm versus 4.3±1.7mm, p-value =0.38*). The average size of tuberculin reactivity was larger in the group with BCG scar than without, the difference was statistically significant (*7.6 ± 1.6 mm versus 2.5±1.3 mm, p-value<0.001*). (Table 1) Scar negative cases were only in two groups who had TST induration less than 5 mm. (n=26) and between 5-9 mm. (n=5). We did not have any scar negative cases in third group (≥10 mm.).

No local reaction and complication at the site of BCG vaccination or development of regional lymphadenopathy was seen.

Table 1. Tuberculin Skin Test and BCG Scar in children 3 months after vaccination at birth in Arak, Iran

TST Size	Number of cases	Scar	
		Positive (≥3 mm)	**Negative (<3mm)**
0-5 mm	309 (39%)	283 (91.5)	**26 (8.5%)**
5-10 mm	436 (55%)	431 (98.8)	**5 (1.2%)**
≥10	49 (6%)	49 (100%)	**0 (0%)**
Total	**794 (100%)**	**763 (96%)**	**41(4%)**

Discussion

In our study, B.C.G scar diameter was ≥3 mm in diameter in 96 % of cases. This proportion is higher than reported in 1998 in Shiraz (71.5%) and in 1991 in the Islamic Republic of Iran (27.2%) in the vaccinated children who had been given the BCG vaccination at birth. (16) Most of the authorities in this field believe that BCG vaccination should result in a long-standing scar in more than 90% of the cases. (16) The differences could be attributed to the type of vaccine, immune response of the children and the method of vaccination. These results are similar to other studies showing low scar failure rates in South Africa, Sri Lanka, and India. (12, 17)

High repeatability of BCG scar measurements with increasing time after vaccination has also been reported before, signifying that the character of the scar stabilizes over time(18, 19) studies have observed that the defining stages in the scar formation process took place particularly during the first 8 weeks, after which the scar stabilized. (12) Our results show that infants who were vaccinated within the first month of life nearly always formed a scar. Thus, a BCG scar was a sensitive marker of the vaccination status.

However, in some studies strongly suggest that failure of formation of BCG-scar at the site of BCG vaccination may not necessarily imply failure of immunization because majority of them do elicit positive in vitro leukocyte migration inhibition (LMI) response. (20) In addition to tuberculin skin test (TST), the Quantiferon test (QFT, based on whole blood γ-interferon release) had been recently proposed. (21)

Another aspect that underscores the importance of assessing BCG scar presence is its relation to TST reactivity. Our results demonstrate about 60% of the children had a TST response >5 mm three months after vaccination at birth. This is consistent with studies that show an association between TST reactions 5 to 9 mm and the presence of a BCG scar. In some studies TST reaction >10 mm among infants has been associated with TB exposure rather than with other factors (eg, age, nutritional status, time since vaccination). (12, 22)

Our results demonstrate that TST reaction size in those with a BCG scar was greater than those without a scar in all the children under study .This was similar to other previous studies carried out in Iran. (16)

In low-income countries, vaccination may not only reduce adverse events but may also save lives. BCG vaccine may have a non-specific beneficial effect on infants mortality in low-income countries, and a positive TST reaction and BCG-scarring have been associated with better infant survival. (23-26) As a final point, in spite of widespread BCG vaccination, the tuberculin skin test can still be used as a useful measure in the epidemiology of tuberculosis.

Conclusion

The present study concludes that about 96 % infants had a successful response to BCG vaccination while 4% did not develop a scar. In addition, about 60% **of** the cases had a TST response >5 mm three months after vaccination at birth. In some countries, repeat vaccination is universal. In others, it is based on either tuberculin skin testing or the absence of a typical scar. Since the absence of development of the scar does not always signify a lack of response to the BCG, therefore it is better to do further immunological tests (e.g. LMIT or INF-γ Assay) to detect a reaction to the vaccine before deciding to revaccinate these children.

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In vitro antibacterial activity of propolis extracts aged 7 and 365 days on 12 different species of bacteria

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Abstract

This research investigated the *in vitro* antibacterial effects of five different preparations of propolis aged 7 and 365 days on 12 species of bacteria classified into four groups according to their pathogenicity. Propolis extracts used in this study exhibited an antibacterial effect on most of the investigated bacterial species. The effect on *Salmonella genus* was negligible. Furthermore, propolis extracted by ether, acetone, toluol and chloroform had a stronger effect compared to propolis extracted by ethanol. Extracts aged 7 days had a slightly better, statistically insignificant, effect than extracts aged 365 days.

Key words: Propolis extracts, antibacterial activity.

Introduction

Propolis, a product of honey bees, has bactericidal and fungicidal activities and is used as an alternative treatment for infections (Ghisalberti, 1979; Grange and Davey, 1990; Margo-Filho and Carvalho, 1994; Hegazi and Abd El Hady, 2001; Hegazi and Abd El Hady, 2002a; Hegazi and Abd El Hady, 2002b; Popova et al., 2004). Takaisi-Kikuni (1994) demonstrated that propolis inhibited the growth of bacteria by preventing division, changing the permeability of cell membrane, causing partial bacteriolysis and inhibiting the synthesis of proteins. Krol et al. (1993) showed that propolis increased the antibacterial effect of penicillin and streptomycin, and that simultaneous application of propolis and antibiotics decreased the necessary dose of antibiotics, their negative side effects and decreased the possibility of resistance to the applied medication. Dim et al. (1992) showed that propolis significantly activated macrophages, while Sudina et al. (1993) and Strehl et al. (1994) concluded that propolis significantly inhibited the activity of lipoxygenase thus reducing the synthesis of prostaglandins. Arvouet-Grand et al. (1993) showed that propolis had no side effects and that allergy to propolis was rare.

The wide range of action of propolis on various microorganisms is the result of combined activities of flavonoids and aromatic compounds. Flavonoids are compounds that dissolve in nonpolar solvents, such as ether, acetone, toluol and chloroform. Today, ethanol is generally the solvent used in the process of extraction of propolis and all published data show the effects of propolis extracts dissolved in ethanol (Hegazi and Abd El Hady, 2001; Hegazi and Abd El Hady, 2002a; Hegazi and Abd El Hady, 2002b; Kouidhi et al., 2010).

Most researches and approaches to the study of propolis, as well as most of therapeutic forms of propolis, have been performed with an ethanol extract. Since ethanol belongs to the group of less polar solvents, by its application as extractant only less polar active substances are extracted, while flavonoids are extracted in minimal quantities or not at all. The final composition of the final solution depends on the solvent applied. Also, it has been noticed that propolis preparations in the course of time lose some of their bactericidal characteristics and effects (Popova et al., 2004).

The basic hypothesis of this study was that the bactericidal and antimicrobial effect of propolis varies depending on the type of solvent used during the extraction, bacterial species and age of the extracted propolis.

The aim of this study was to determine which propolis solution had most antimicrobial activity in different bacterial cultures. Bactericidal activity of propolis extracted by various solvents: ethanol, ether, acetone, toluol and chloroform of different ages (7 and 365 days) on cultures of 12 different

species of microorganisms (*Morganella morgani, Streptococcus faecalis, Achromobacter xylosoxidans, Sarcina lutea, Escherichia coli, Aeromonas hydrofila, Salmonella Typhimurium, Bacillus subtilis, Salmonella Gallinarum* and *Salmonella Choleraesuis, Staphylococcus aureus, Bacillus cereus*) in neutral environment conditions (pH=7) was investigated.

Materials and methods

In this research standardized pure cultures of bacterial strains procured from the Faculty of Veterinary Medicine, University of Belgrade, were used. Microorganisms were chosen according to the frequency of published researches and frequency of infections in humans. They were classified according to "Bergey's Manual of Determinative Bacteriology" (Tortora et al., 2004). Pure cultures of bacteria belonging to four different groups were used:

- Group I - bacteria banal: *Proteus morgani (morganella), Streptococcus faecalis, Achromobacter xylosoxidans, Sarcina lutea* and *Escherichia coli.*
- Group II - opportunistic pathogenic bacteria: *Aeromonas hydrophila, Salmonella Typhimurium and Bacillus subtilis.*
- Group III - infectious pathogenic bacteria: *Salmonella Gallinarum* and *Salmonella Choleraesuis.*
- Group IV - exotoxic pathogenic bacteria: *Staphylococcus aureus* and *Bacillus cereus.*

To ensure the homogeneity of the solution, only propolis from one colony and a fixed time period was used in this study.

Five solvents were used to extract the active ingredients in propolis: ethanol (C_2H_5OH), ether (C_2H_5-O-C_2H_5), acetone [$(CH_3)_2CO$], chloroform ($CHCl_3$) and toluol ($C_6H_5CH_3$). The entire quantity of extracted propolis was divided into two categories: propolis aged 7 days and propolis aged 365 days. The same experiments were performed with all test microorganisms. Extraction was performed in the following manner, regardless of the type of solvent. The mix of solvent and water (volume ratio from 60:40 up to 96:4) was poured into a mixer with a double container (used for cooling purposes). A detailed description of the extraction process was presented in our other paper (Ivancajic et al., 2010). These solutions were kept in dark brown, glass bottles in a dark place at room temperature for the next 7, or 365 days.

Test microorganisms were cultivated in a nutritious broth. The broth contained peptones, beef extract, sodium chloride and potassium phosphate in a standardized proportion. The dissolved base - broth was heated to boiling point until completely dissolved. The dissolved base, having a pH value of 7.3, was poured into test tubes and sterilized in an autoclave for 15 minutes at 120 °C. Broths cultivated by test microorganisms were incubated for 24 hours at 30 °C. Each test microorganism was re-cultivated in this manner three days in succession before the experiment. In this experiment agar was used as the growth medium. This medium altogether consisted of peptone, beef extract, potassium phosphate and agar in a standardized proportion. 41.3 g of agar powder was suspended in 1000 mL of cold distilled water and left for 15 minutes. The medium was then carefully heated to boiling point so that it could be completely dissolved and poured into bottles and sterilized in an autoclave for 15 minutes at 120 °C. After sterilization the pH of the medium was adjusted to 7, by the addition of 0.1 mol of hydrochloric acid solution or 0.1 mol of sodium hydroxide solution. The melted nutritious medium was then divided into several parts. After cooling to 45-50 °C each part was cultivated by one of the test microorganisms.

The cultures of all 12 test microorganisms were diluted tenfold (down to 0.1 mol) by the addition of sterilized physiological solution and cultivated on 100 mL of nutritious agar medium that had been melted and cooled to 45-50 °C. The cultivated agar was poured into plastic, sterile Petri dishes, volume 100 mL, so that the thickness of the base was 2 mm. Three holes, 10 mm in diameter, were drilled in the set agar in each Petri dish.

Antimicrobial activity of the propolis samples was investigated by the method of growth inhibition of the chosen test microorganism in the culture medium. Various propolis extracts were placed in the holes made in the cultivated growth medium from which it could freely diffuse into the environment. The growth of the investigated bacterium was inhibited in the diffusion zone according to its sensitivity to propolis. The width of this inhi-

bition zone showed the degree of sensitivity of the investigated bacterium to propolis, ranging from a narrow or nonexistent inhibition zone, in the case of resistance, to a wider zone, corresponding to certain degrees of sensitivity.

The measuring procedure for antimicrobial activity of propolis was:

Propolis extracts were heated in a water bath at 50 °C until a semi-liquid consistency was reached. Each sample was subsequently poured into holes made in the nutritious agar base. The prepared holes were completely filled by the propolis extracts. The cultures were then incubated for 2 hours at 4 °C, followed by 18 hours at 30 °C. The width of the inhibition zone of growth of the tested microorganism was measured from the margin of the hole to its outer border.

The value, expressed in millimeters, was the mean value of measurements around all three holes in one Petri dish.

In order to determine the level of differences between the arithmetical means of two samples (activity of propolis aged 7 and 365 days) Student T-test (Janosevic et al., 2000) was used. Modification of the Student T-test according to Bonferroni (Janosevic et al., 2000) was used to mutually compare multiple means in one statistical sample (propolis extracted in 5 different of solvents). The p values <0.05; <0.01 and <0.001 were considered to be significant.

Results

All results have been statistically processed and shown in tables. Each investigated parameter is represented by the mean value ± standard error (SE) and statistical significance, separately marked.

Results of the inhibitory effect of 5 preparations of propolis aged 7 and 365 days on bacteria from group I:

1. Morganella morgani

Table 1 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Morganella morgani* in neutral cultures (pH=7).

It was clear that between propolis extracted in various solvent, regardless of the age, exist a statistically significant difference considering inhibitory effect on the growth of the bacterium *Morganella morgani* ($p<0.001$). Propolis extracted by ether aged 7 days and propolis extracted by acetone (365 days) showed the strongest inhibitory effect ($p<0.05$).

There was a statistically significant decreasing effect ($p<0.01$) of propolis extracted in ether aged 365

Table 1. Activity of the propolis solution aged 7 and 365 days on Morganella morgani in neutral cultures (ph=7).

Morganella morgani					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	6.60 ± 0.35	7.30 ± 0.27*	7.10 ± 0.50	4.30 ± 0.25	5.00 ± 0.27
365	6.60 ± 0.20	6.60 ± 0.42	7.00 ± 0.35*	6.30 ± 0.17	5.30 ± 0.32

*(7) - *p<0.05 vs other groups, except acetone*
*(365) - *p<0.05 vs other groups, except ether*

Table 2. Activity of the propolis solution aged 7 and 365 days on Streptococcus faecalis in neutral cultures (ph=7).

Streptococcus faecalis					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	3.30 ± 0.10	3.00 ± 0.29	5.00 ± 0.42*	5.00 ± 0.39*	2.00 ± 0.25
365	4.00 ± 0.27	3.30 ± 0.18	6.30 ± 0.30*	1.55 ± 0.35	4.60 ± 0.15

*(7) - *p<0.05 vs other groups, except acetone (toluol)*
*(365) - *p<0.05 vs other groups*

days in comparison to 7 days extract. There was also a statistically significant increasing effect ($p<0.001$) of propolis extracted in toluol aged 365 days.

2. Streptococcus faecalis

Table 2 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Streptococcus faecalis* in neutral cultures (pH=7).

The effect of propolis extracted in acetone, aged 365 days was most pronounced and statistically significant in comparison to other groups. There was a statistically significant effect ($p<0.05$) of propolis extracted in toluol aged 7 days in comparison to other groups (except acetone).

3. Achromobacter xylosoxidans

Table 3 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Achromobacter xylosoxidans* in neutral cultures (pH=7).

Comparing the effects of various propolis preparations it was apparent that propolis extracted in toluol aged 7 days had the most distinct and statistically significantly ($p<0.05$) greater inhibitory effect on the growth of the bacterium *Achromobacter xylosoxidans.* There was also a statistically significant effect ($p<0.05$ vs other groups) of propolis extracted in ethanol aged 365 days.

In comparison to 365 days, there were found a statistically better effects of propolis extracted in ether ($p<0.05$) and toluol ($p<0.001$).

4. Sarcina lutea

Table 4 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Sarcina lutea* in neutral cultures (pH=7).

The most significant effect was seen using propolis extracted in toluol and aged 7 days

Table 3. Activity of the propolis solution aged 7 and 365 days on Achromobacter xylosoxidans in neutral cultures (ph=7)

Achromobacter xylosoxidans					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	6.60 ± 0.30	7.30 ± 0.21	7.30 ± 0.24	9.00 ± 0.51*	6.00 ± 0.02
365	7.60 ± 0.22*	6.30 ± 0.29	6.60 ± 0.31	5.30 ± 0.32	6.00 ± 0.18

*(7) - *p<0.05 vs other groups*
*(365) - *p<0.05 vs other groups, except acetone*

Table 4. Activity of the propolis solution aged 7 and 365 days on Sarcina lutea in neutral cultures (ph=7)

Sarcina lutea					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	6.30 ± 0.24	5.60 ± 0.18	7.60 ± 0.22	9.30 ± 0.45*	5.80 ± 0.25
365	6.60 ± 0.19*	6.50 ± 0.21	4.60 ± 0.34	4.50 ± 0.37	6.00 ± 0.32

*(7) - *p<0.05 vs other groups*
*(365) - *p<0.05 vs acetone and toluol*

Table 5. Activity of the propolis solution aged 7 and 365 days on Escherichia coli in neutral cultures (ph=7)

Escherichia coli					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	6.30 ± 0.28	6.30 ± 0.18	7.30 ± 0.21	8.60 ± 0.38*	6.60 ± 0.24
365	6.00 ± 0.31	7.30 ± 0.21*	6.00 ± 0.27	5.50 ± 0.22	6.00 ± 0.28

*(7) - *p<0.05 vs other groups*
*(365) - *p<0.05 vs other groups*

(p<0.05). There was a statistically significant effect (p<0.05) of propolis extracted in ether, aged 365 days versus acetone and toluol groups.

In comparison to 365 days aged solutions, there were a statistically better effect (p<0.001) of propolis extracted in acetone and toluol (p<0.001) aged 7 days.

5. *Escherichia coli*

Table 5 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Escherichia coli* in neutral cultures (pH=7).

Propolis extracted in toluol aged 7 days had the most distinct and statistically significantly (p<0.05) greater inhibitory effect on the growth of *Escherichia coli* compared to the other propolis extracts.

There was a statistically better effect (p<0.05) of propolis extracted in ether, aged 365 days.

In comparison to 365 days aged solutions, there was a statistically better effect (p<0.05) of propolis extracted in acetone aged 7 days. There was also a statistically better effect (p<0.001) of propolis extracted in toluol aged 7 days.

Results of the inhibitory effect of 5 preparations of propolis aged 7 and 365 days on bacteria from group II:

1. Aeromonas hydrophila

Table 6 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Aeromonas hydrophila* in neutral cultures (pH=7).

The results showed that all propolis extracts had a moderate inhibitory activity on the growth of the bacterium *Aeromonas hydrophila*.

There was a statistically better effect (p<0.05 versus all other groups) of propolis extracted in toluol aged 7 days. There was a statistically better effect (p<0.05) of propolis extracted in ethanol aged 365 days.

Except toluol group, 365 days aged solutions exerted statistically better effect (p<0.05 - p<0.001) tnan corresponsing 7 days aged solutions.

Table 6. Activity of the propolis solution aged 7 and 365 days on Aeromonas hydrophila in neutral cultures (ph=7)

Aeromonas hydrophila					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	3.30 ± 0.42	2.60 ± 0.34	4.30 ± 0.27	6.00 ± 0.36*	2.00 ± 0.28
365	6.30 ± 0.32*	4.30 ± 0.28	5.30 ± 0.30	2.00 ± 0.31	5.30 ± 0.26

*(7) - *p<0.05 vs other groups*
*(365) - *p<0.05 vs ether and toluol*

Table 7. Activity of the propolis solution aged 7 and 365 days on Salmonella Typhimurium in neutral cultures (ph=7)

Salmonella Typhimurium					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	0	0	0	0	0
365	0	0	2.00 ± 0.16	0	1.00 ± 0.22

Table 8. Activity of the propolis solution aged 7 and 365 days on Bacillus subtilis in neutral cultures (ph=7)

Bacillus subtilis					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	6.00 ± 0.37	6.30 ± 0.32	5.60 ± 0.22	7.30 ± 0.29*	5.00 ± 0.33
365	5.60 ± 0.32	6.30 ± 0.28*	4.60 ± 0.26	4.30 ± 0.30	5.00 ± 0.35

*(7) - *p<0.05 vs other groups, except ether*
*(365) - *p<0.05 vs other groups, except ethanol*

2. Salmonella Typhimurium

Table 7 show mean values of inhibition zone of propolis aged 7 and 365 days on bacterial cultures of *Salmonella Typhimurium* in neutral cultures (pH=7).

The inhibitory effect of all preparations of propolis on the growth of the bacterium *Salmonella Typhimurium* was irrelevant. A mild inhibitory activity on the growth of *Salmonella Typhimurium* was shown by propolis extracted in acetone, aged 365 days and propolis extracted in chloroform, aged 365 days, however these effects were statistically insignificant.

3. Bacillus subtilis

Table 8 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Bacillus subtilis* in neutral cultures (pH=7).

Propolis extracted in toluol aged 7 days had the most distinct and statistically significantly ($p<0.05$) greater inhibitory effect on the growth of *Bacillus subtilis* compared to other extracts of propolis.

There was a statistically better effect ($p<0.05$ compared to other groups, except ethanol) of propolis extracted in ether, aged 365 days.

In comparison to 365 days aged solution, there was a statistically better effect ($p<0.001$) of propolis extracted in toluol aged 7 days.

Results of the inhibitory effect of 5 preparations of propolis aged 7 and 365 days on bacteria from group III:

1. Salmonella Gallinarum

Table 9 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Salmonella Gallinarum* in neutral cultures (pH=7).

These results clearly showed that the inhibitory effect of all types of propolis on the growth of *Salmonella Gallinarum* was irrelevant. Namely, the inhibitory effect of propolis was zero, except for propolis extracted in acetone aged 365 days and propolis extracted in chloroform aged 365 days.

2. Salmonella Choleraesuis

Table 10 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Salmonella Choleraesuis* in neutral cultures (pH=7).

The inhibitory effect of all extracts of propolis on the growth of *Salmonella Choleraesuis* was irrelevant. The inhibitory effect of propolis was zero, except for propolis extracted in acetone aged 365 days.

Results of the inhibitory effect of 5 preparations of propolis aged 7 and 365 days on bacteria from group IV:

1. Staphylococcus aureus

Table 11 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Staphylococcus aureus* in neutral cultures (pH=7).

Table 9. Activity of the propolis solution aged 7 and 365 days on Salmonella Galinarum in neutral cultures (ph=7)

Salmonella Galinarum					
Days	$\overline{X} \pm SE$				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	0	0	0	0	0
365	0	0	2.00 ± 0.47	0	1.30 ± 0.24

Table 10. Activity of the propolis solution aged 7 and 365 days on Salmonella Choleraesuis in neutral cultures (ph=7)

Salmonella Choleraesuis					
Days	$\overline{X} \pm SE$				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	0	0	0	0	0
365	0	0	1.30 ± 0.28	0	0

Table 11. Activity of the propolis solution aged 7 and 365 days on Staphylococcus aureus in neutral cultures (ph=7).

Staphylococus aureus					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	4.60 ± 0.19	4.30 ± 0.21	5.00 ± 0.37*	5.00 ± 0.28*	3.30 ± 0.24
365	5.00 ± 0.15	5.00 ± 0.24	5.00 ± 0.28	4.60 ± 0.27	4.60 ± 0.22

*(7) - *p<0.05 vs chloroform*
(365) – no significant differences

Table 12. Activity of the propolis solution aged 7 and 365 days on Bacillus cereus in neutral cultures (ph=7)

Bacillus cereus					
Days	$\overline{X}$ ± SE				
	ethanol	**ether**	**acetone**	**toluol**	**chloroform**
7	5.60 ± 0.28	6.60 ± 0.17	7.30 ± 0.31	9.00 ± 0.42*	5.00 ± 0.39
365	6.60 ± 0.26	5.30 ± 0.21	4.00 ± 0.29	3.60 ± 0.28	7.30 ± 0.3*

*(7) - *p<0.05 vs other groups*
*(365) - *p<0.05 vs other groups, except ethanol*

The most distinct inhibitory effect was that of propolis extracted by acetone and toluol (7 days aged).

The same results were seen in ethanol, ether and acetone groups (365 days aged). In comparison to 7 days aged solution, there was a statistically better effect ($p<0.01$) of propolis extracted in chloroform aged 365 days.

2. *Bacillus cereus*

Table 12 show mean values of inhibition zones of propolis aged 7 and 365 days on bacterial cultures of *Bacillus cereus* in neutral cultures (pH=7).

The results showed that all preparations of propolis had an inhibitory effect on the growth of the bacterium *Bacillus cereus*. The best effect ($p<0.05$ versus other groups) showed propolis extracted by toluol, aged 7 days. There was also a statistically better effect ($p<0.05$ compared to other groups, except ethanol) of propolis extracted in chloroform aged 365 days.

In comparison to 7 days aged solutions, the smallest inhibitory activity were noticed in acetone and toluol groups aged 365 days ($p<0.001$).

Discussion

The effects of various types of propolis were tested in this research, i.e. the effect of propolis extracted in different types of solvents (ethanol, ether, acetone, toluol and chloroform), 7 and 365 days old, on twelve species of bacteria which were classified into four groups according to their pathogenicity.

The results of this research showed that propolis had a distinct anti-bacterial effect and were a confirmation of many previous researches, such as the results of Grange and Davey (1990), which showed that propolis had an anti-bacterial effect, especially on Gram-positive bacteria, while the effect was weaker on Gram-negative bacteria.

Serkedjieva et al. (1992), Dumitrescu et al. (1992) and Amoros et al. (1994) had similar results in their studies. These were also in accordance with the results from several clinical studies conducted in the countries of the former Soviet Union (Tsarev et al. 1985), Romania (Esanu, 1981), and China (Pang and Chen, 1985). All these studies independently showed the anti-bacterial and anti-infective effect of propolis.

In the sources available to us, we had not come across the results of testing the anti-bacterial effect of propolis on all bacterial species as in our study (regardless of the type of solvent used); therefore,

it is impossible to make all the comparisons. If in our results we analyze the effect of all types of propolis on bacteria from group I, the banal bacteria group, it is clear that propolis showed the best anti-bacterial effect on *Escherichia coli*, followed by *Achromobacter xylosoxidans*, *Sarcina lutea* and *Morganella morgani*, while there was least effect on *Streptococcus faecalis*. The inhibitory effect of propolis on the growth of *Streptococcus faecalis* was not negligible; it was just weaker than on the other types of bacteria of this group. These results were in full accordance with the results of Stepanovic et al. (2003), which showed that propolis had significant anti-bacterial activity on Gram-positive bacteria, and that *Streptococcus faecalis* was the most resistant Gram-positive bacteria.

The anti-bacterial effect of propolis was generally tested on *Escherichia coli*. The results obtained by Simuth et al. (1986), Brumfitt et al. (1990), Drago et al. (2000) and Sforcin et al. (2000) were in full accordance with the results from this study, which confirmed a very significant inhibitory effect of propolis on the growth of *Escherichia coli*.

The analysis of the inhibitory effect of all types of propolis on bacteria from group II, the opportune pathogenic bacteria, clearly showed that propolis exhibited the best anti-bacterial effect on *Bacillus subtilis*, a lesser one on *Aeromonas hydrophila*, and the least on *Salmonella Typhimurium*.

Results very similar to our results for the anti-bacterial effect of propolis on *Bacillus subtilis* were obtained by Brumfitt et al. back in 1990 (Brumfitt et al., 1990), and Pepeljnjak et al. (1985).

It was evident that the effect of all kinds of propolis on bacteria from group III, the infectious pathogenic bacteria, i.e. *Salmonella Gallinarum* and *Salmonella Choleraesuis* was negligibly small. Our results differed from those of Okoneko (1986), who showed that propolis inhibited the activity of free radicals created in the process of oxidation of lipids during salmonellosis. The differences were probably a consequence of application of different methods of testing.

Our results clearly showed that no matter what kind of solvent was used, propolis had a very weak, in fact, negligible inhibitory effect on the growth of the bacteria from *Salmonella* genus, regardless of whether they belonged to the opportune pathogenic bacteria group, such as *Salmonella Typhimurium*, or the infectious pathogenic bacteria group, such as *Salmonella Gallinarum* and *Salmonella Choleraesuis.*

The analysis of the inhibitory effect of all types of propolis on bacteria from group IV, the infectious pathogenic bacteria group, clearly showed that propolis exhibited a significant anti-bacterial effect on *Staphylococcus aureus*, and a lesser one on *Bacillus cereus*.

Very similar results for the anti-bacterial effect of propolis on *Staphylococcus aureus* were published by Brumfitt et al. (1990), Qiao and Chen (1991), Krol et al. (1993), Drago et al. (2000), Sforcin et al. (2000), as well as Onlen et al. (2007).

The results from this wide research showed that propolis extracted in ether, acetone, toluol and chloroform had the best inhibitory effects on the growth of the examined bacteria, except for the bacteria from *Salmonella* genus.

The inhibitory effect of those propolis extracts was statistically very significant, because the mean values of bacteria growth inhibition zones varied from 7.6 to 12 mm, which represented an almost complete growth inhibition of these bacteria.

The results of this research without doubt show that propolis extracted in toluol and chloroform (inhibition zone width over 8 mm) had the best inhibitory effects on the following bacteria: *Morganella morgani, Achromobacter xylosoxidans, Sarcina lutea, Escherichia coli, Bacillus subtilis* and *Bacillus cereus.*

Based on these results, it is possible to apply propolis in an appropriate solvent in cases of a tested bacteria infection with a positive outcome. In this way, the application of propolis would be targeted, and more successful. In cases of hypersensitivity or resistance to antibiotics, propolis could substitute their application.

When the inhibitory effect of all tested types of propolis was analyzed, it was obvious that on most bacteria tested in this study propolis aged 7 days had a statistically negligible better effect than propolis aged 365 days, except for the inhibitory effect of propolis extracted in toluol and aged 7 days on the growth of bacteria *Streptococcus faecalis*, *Achromobacter xylosoxidans*, *Escherichia coli, Aeromonas hydrophila, Bacillus subtilis and Bacillus cereus.* Furthermore, this statistically negligible

better effect was not related to the inhibitory effect of propolis extracted in acetone and toluol aged 7 days on the growth of the bacterium *Sarcina lutea.*

It was clear that in seven cases propolis extracted in toluol and aged 7 days and in two cases propolis extracted in acetone aged 7 days had a statistically significantly better effect ($p<0.001$) than the same type of propolis aged 365 days.

In further research, these results should be checked, to see whether the better effect of propolis extracted in acetone and toluol 7 days old is a consequence of the toxic effects of the solvents, whose effect diminishes after a longer period of time.

Based on all these results, it is possible to take a step further in applying propolis. Namely, the solvents used to extract propolis, such as acetone, toluol and chloroform, fall into the group of toxic matters, which could have harmful effects on human organism even if ingested in smaller amounts.

For that reason, the obtained extracts of propolis dissolved in such solvents should be evaporated in vacuum evaporators and transformed into a solid state, i.e. powder, which contains only active components of propolis. The subsequent research is going to be directed that way.

Conclusion

Based on our results it can be concluded that:

Propolis when extracted in ethanol, ether, acetone, toluol and chloroform exhibits anti-bacterial effect on group I bacteria - banal bacteria (*Proteus morgani, Streptococcus faecalis, Achromobacter xylosoxidans, Sarcina Lutea* and *Escherichia coli*).

Group II bacteria – opportune pathogenic bacteria (*Aeromonas hydrophila and Bacillus subtilis)* are significantly sensitive to the effect of propolis extracted in all types of solvents. *Salmonella Typhimurium* - opportune pathogenic bacteria, is insensitive to the effect of propolis, no matter what solvent is used for extraction.

Group III bacteria - infective pathogenic bacteria (*Salmonella Gallinarum and Salmonella Choleraesuis*) are relatively insensitive to the effect of propolis.

Propolis extracted in all types of solvents exhibits a significant anti-bacterial effect on group IV bacteria - exotoxic pathogenic bacteria (*Staphylococcus aureus* and *Bacillus cereus*).

Propolis extracted in ether has the most pronounced inhibitory effect on *Proteus morgani (Morganella morgani)* and *Aeromonas hydrophila.*

Propolis extracted in acetone exhibits the most intense anti-bacterial effect on *Streptococcus faecalis, Aeromonas hydrophila* and *Staphylococcus aureus*.

Propolis extracted in toluol exhibits a significant anti-bacterial effect on *Morganella morgani, Achromobacter xylosoxidans, Sarcina lutea, Escherichia coli, Bacillus subtilis* and *Bacillus cereus.*

Propolis extracted in chloroform exhibits a significant antibacterial effect on *Escherichia coli.*

The age of propolis had no significant influence on its anti-bacterial effect.

The application of propolis by using these results would give much better therapeutic effects, whether used as a medicine of choice or in combination with a suitable antibiotic depending on the severity of the disease.

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Hematological parameters in patients with mitral regurgitation secondary to idiopathic chordae tendineae rupture

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Abstract

The aim of this study is to investigate the blood characteristics in patients with severe mitral regurgitation due to idiopathic rupture of chordae tendinea.

26 patients who diagnosed severe mitral regurgitation due to idiopathic chordae tendineae (group 1) and 40 healthy age-sex matched subjects (group 2) were chosen as control group. All the echocardiography and clinical data were evaluated retrospectively between 2007 and 2012.

There was not statistically significant difference in age, height, hypertension, diabetes and medications being used between the two groups. But, patients with idiopathic chordae tendineae rupture had more overweight than the controls (74.9±7.4 kg versus 70.7±7.5, and

P=0.031). Also platelets, mean platelet volume, hemoglobin, hematocrit, lymphocytes, monocytes, eosinophil leucocytes, mean corpuscular hemoglobin concentration, red blood cell distribution width, mean corpuscular volume, plateletcrit, and platelet distribution width did not differ between the two groups. Red blood cell, neutrophil and basophil counts were significantly higher in patients with idiopathic chordae tendineae rupture (rbc, 42.8±5.05 versus 42.8±5.05, p=0.022; neu, 45.8±14.99 versus 38.3±11.70, p=0,026 and baso, 0.5±0.50 versus 0.8±0.33, p=0.002)

This study showed that, patients with idiopathic chorda tendinea rupture is more overweight and their red blood cell, neutrophil and basophil counts are higher than controls. Those results suggest inflammation in the etiology of idiopathic chorda tendinea rupture.

Key words: Idiopathic chorda tendinea rupture, blood characteristics, neutrophil, basophil, platelets.

Introduction

Rupture of mitral chorda tendineae (RCT) was first described in 1806 by Corvisart[1]. RCT is an important cause of mitral regurgitation and its prevalence is increasingly being reported. Majority of the patients with RCT present to cardiology and emergency departments by cardiogenic shock or pulmonary edema due acute severe mitral regurgitation. Clinical symptoms depend and therapeutic decision is made according to the severity of chordae rupture, mitral regurgitation degree and associated cardiac disease. But majority of the patients with RCT eventually has a progressive course which may require mitral valve surgery[2, 3]. There are known varied etiologies of RCT such as ischemia, infective endocarditis, rheumatic heart diseases and, less frequently Kawasaki disease, blunt chest trauma, acute rheumatic fever, connective tissue diseases and left ventricular volume overload causing cord stretch[4-10]. Nearly half of the RCT has not any known etiology[4].

Chronic inflammation may be associated with the pathophysiology of spontaneous RCT as in the abdominal aorta, degenerative aortic stenosis, rheumatic heart disease[11, 12]. Previous studies showed that, markers of inflammation such as high sensitive C-reactive protein, tumor necrosis alpha and monocyte chemo attractant protein-1 was increased in patients with heart valve pathology[13]. The role of the inflammation has been discussed in those studies associated with structural element of the mitral valve such as chordae tendineae**.** Limited number of studies on this subject has showed that inflammation with mechanical stress and hypoxia effective in the development of chordae tendineae rupture[14-16].

This study aimed to investigate the distribution of hematological indices in patients with mitral regurgitation due to idiopathic RCT.

Materials and methods

All the echocardiography and clinical data were evaluated retrospectively between 2007 and 2012. 26 patients who diagnosed idiopathic RCT (group 1) and 40 healthy age-sex matched subjects (group 2) were chosen as control group.

Patients with sub-acute endocarditis, rheumatic heart disease, mitral valve prolapse, myxomatous degeneration, connective tissue disease, blunt chest trauma, hypertrophic cardiomyopathy, non-heart mitral valve disease, ischemic heart disease, myocardial infarction, chronic renal failure, chronic-active inflammatory disease and smokers were excluded from the study. Any patient who required cardiac surgery due to hemodynamic disturbances was also excluded. Clinical and demographical data such as age, gender, height and weight were recorded on patient charts. Diabetes mellitus and hypertension, drug use were evaluated.

Platelet counts(plt), mean platelet volume (mpv), red blood cell (rbc), neutrophil (neu), basophil (baso)hemoglobin (hgb), hematocrit (hct), lymphocites (lym), monocytes (mono), eosinophils (eos), mean corpuscular hemoglobin concentration (mchc), red blood cell distribution width (rdw), mean corpuscular volume (mcv), plateletcrit (pct), and platelet distribution width (pdw) were collected in all patients as hematological indices.

Echocardiographic examination

M-mode and 2D images, spectral and color flow Doppler recordings of all patients were obtained with Vivid 3 echocardiography device, using 3.5 MHz probe (General Electric, Haifa, Israel). Transesophageal echocardiography (TEE) was performed in all patients with mitral valve regurgitation. All RCT was diagnosed by TEE.

All patients underwent standard two-dimensional and Doppler echocardiographic examinations with detailed evaluation of heart function. Imaging planes were standardized, and they included the parasternal left heart long-axis view, the aortic and MV short-axis view, and the apical four- and two-chamber views.

Left atrial (LA) diameter was measured from the parasternal left heart long-axis view. Pulmonary artery trunk and pulmonary flow were measured from the aortic short-axis view. We also measured mitral inflow, including the E velocities and aortic valve flow. Pulmonary systolic pressure was calculated according to velocity of tricuspid regurgitation by the Bernoulli equation. The left ventricular end-diastolic diameter (LVEDd) and ejection fractions (EF) were calculated by the M-mode method.

Valvular regurgitation was graded as: mild (I), which was defined as MR jets with an area < 20% of the LA area; moderate (II) as 20-40% of the LA area; and severe (III) as > 40% of the LA area. Mild pulmonary artery hypertension was defined as a pressure of 36 to 51 mmHg.

TEE exams were usually conducted using a GE vivid 3 with a 12 MHz multiplane transesophageal transducer. The MV and its chordae tendineae were observed in the left ventricular midesophageal and MV transgastric views, with rotation of the TEE probe to achieve the clearest view (17).

Statistical analysis

All data were analyzed by SPSS (Statistical Package for Social Sciences) for Windows 16.0 program. The non-parametric datas were expressed as percent (%) and parametric datas were expressed as ± mean standard deviation. "Student-t" test was used to compare numerical datas, "Chi-square" test was used to compare of categorical datas between groups. $p < 0.05$ was considered significant for statistical analyzes.

Results

There was not statistically significant difference between the two groups in age, height, hypertension, diabetes and medications being used. But, patients with idiopathic RCT has more overweight than the controls (74,9±*7,4 kg versus 70,7±7,5, and P=0.031) (Table 1). Also,* plt, mpv, hgb, hct, lym, mono, eos, mch, mchc, rdw, mvc, pct ve pdw did not differ between the two groups. Rbc, neu and baso counts were significantly higher in patients with idiopathic chordae tendineae rupture (rbc, 42.8±5.05 versus 42.8±5.05, p=0.022; neu, 45.8±14.99 versus 38.3±11.70, p=0.026 and baso, 0.5±0.50 versus 0.8±0.33, p=0.002) (Table 2).

Tabel 1. Clinical and demographic characteristics of patients and control group

	Chordal Rupture, N: 26	Control Group, N: 40	P value
Age (year)	60,9±12	61.9±5,9	0.651
Height (cm)	167,8± 6,9	166,5±6,60	0.419
Weight (kg)	74,9±7,4	70,7±7,5	0.031
Hypertension %	43,5	45	0.089
Diabetes %	26	28	0.078

Table 2. Hematological indices in patients and control group

Data	Chordal Rupture, N: 26	Control Group, N: 40	P value
Wbc (K/uL)	75,7±16	68,2±14	0,056
Rbc (M/uL)	42,8±5,05	42,8±5,05	0,022
Hgb (g/dl)	123,1±18,3	129,5±10,4	0,076
Hct (%)	369,3±53,3	387,6±31,3	0,083
Plt (K/uL)	260,9±80,6	271,5±52,3	0,518
Mch (pg)	287,3±19,7	281,3±44,8	0,520
Mchc (g/dl)	333,3±10,4	327,8±48,6	0,578
Rdw (%)	163,23±43,34	152,05±13,8	0,133
Mcv (fl)	862,2±57,2	854,4±43,4	0,530
Mpv (fl)	75,6±11,5	78,6±11,4	0,298
Pct (10(GSD))	1,9±0,8	2,1±0,5	0,146
Pdw (%)	176,9±12	176,6±8,7	0,923
Neu (K/uL) (%)	597±97,4	555±76,36	0,055
Neu (K/uL)	45,8±14,990	38,3±11,70	0,026
Lym (K/uL) (%)	297±90,24	333±67,88	0,069
Lym (K/uL)	22±7,6	22±5,1	0,945
Mono (K/uL) (%)	76±24,9	74±21,1	0,758
Mono (K/uL)	5±2,1	5±1,4	0,406
Eos (K/uL) (%)	18±19,4	23±19,04	0,297
Eos (K/uL)	1,5±1,5	1,6±1,2	0,777
Baso (K/uL) (%)	8,1±3,4	10,2±3,6	0,024
Baso (K/uL)	0,5±0,50	0,8±0,33	0,002

Abbreviations: Plt: platelet, Mpv: mean platelet volume, Hgb: hemoglobin , Hct: hematocrit, Lym: lymphocite, Mono: monocytes, Eos: eosinophils, Rbc: red blood cell, Neu: neutrophil, Baso: basophil, Mch: mean corpuscular hemoglobin, Mchc: mean corpuscular hemoglobin concentration, Rdw: red blood cell distribution width, Mcv: mean corpuscular volume, Pct: plateletcrit, Pdw: platelet distribution width.

Discussion

Mitral valve regurgitation can develop as a result of any disorder which occurred in any of the cardiac structures (mitral annulus, mitral leaflets, chordae tendineae, and papillary muscle). Chordal rupture is one of the most important causes of acute mitral regurgitation[18, 19]. Rupture of a single structure of chordae will be limited hemodynamic effects and generally do not require treatment; rupture of a multiple structure of chordae causes, life-threatening and require emergency surgical repair acute severe mitral regurgitation[20-21].

Mitral chordae rupture is more common in men over the age of 50[1]. In the literature, blunt thoracic trauma, connective tissue diseases, coronary artery disease, subacute infective endocarditis, rheumatic mitral stenosis, mitral valve prolapse was found as the underlying cause. Undetermined cases are defined as primary (idiopathic) chordal rupture in etiology. The chordae tendineae rupture rate of 51.2% is primary according to a recent systematic review[4].

Mechanism predisposing to rupture in patients with primary chordae rupture is still unknown. In earlier studies inflammation was found to play a

role in the pathophysiology of spontaneous rupture of the Achilles tendon and the abdominal aorta have a similar fibrous structure of chordae tendineae[2, 22]. From there, the limited number of studies was found inflammation, angiogenesis and matrix metalloproteinase activation effective in formation of chordal rupture similary [2, 4, 23, 24].

Tenomodulin which is an anti-angiogenic factor was not found in the samples of the surgically severed chorda and activation of matrix metalloproteinases, some portion produced by neutrophils, seems to have increased in the study made by Kimura and friends. Alteration of these factors has been shown to cause degeneration of chordae, by increasing the formation of abnormal blood vessels. This study also investigated for the absence of tenomodulin. Tenomodulin levels were decreased significantly after hypoxic incubation or mechanical stres applied to chordae tendineae interstitial cells in vitro environment. From there, mechanical stresses such as hypertension or hypoxia at tissue level are thought to may play a role in the development of chordal rupture [25].

Decreases in the levels of oxygen pressure in tissue are known to increase erythropoietin levels; therefore levels of erythrocyte[26, 27]. In our study Rbc levels were found significantly higher in patients with primary chordae rupture. This, as in previous studies suggest that tissue hypoxia play a role in the pathophysiology of cord rupture[4, 9, 12].

According to some studies obesity effects on left ventricular mass more than high blood pressure[28]. In our study, the group of primary chordae tendineae rupture was found to be overweight significantly than the control group. According to this result, we can say that obesity is a risk factor for the rupture of the chordae tendineae and mitral valve diseases. But significantly overweight is beyond the scope of this study. Mitral valve prolapse has been shown to as a result myxomatous degeneration and also chronic inflammation in a study by Takanabu and friends. Valves of these patients were examined; histologically extensive scar formation and chronic inflammatory cell such as lymphocytes and plasma cells infiltration was determined. Some of these patients, defined as post inflammatory mitral valve prolapse, were observed that spontaneous chordal rupture[29].

As a result of these studies; inflammation has provided strong evidence, playing an active role in the pathophysiology of primary chordae rupture. In our study, we think that, the increase in the levels of neutrophils and basophils in the patients are as a result of local inflammatory response.

Our study showed fort the first time that, patients with idiopatic chorda tendinea ruptue is more overweight and their rbc, neu and baso counts are higher than controls. Those results suggest the inflammation in the etiology and pathogensis in idiopathic chorda tendinea rupture.

Conclusion

Results of this study showed that, patients with idiopathic chorda tendinea rupture is more overweight and their rbc, neu and baso counts are higher than controls. Those results suggest inflammation in the etiology of idiopathic chorda tendinea rupture.

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Tracing contacts of TB patients in Malaysia: Costs and practicality

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Abstract

Background: Tuberculin skin testing (TST) and chest X-ray are the conventional methods adopted for tracing a tuberculosis (TB) suspects. The purpose of the study was to calculate the cost incurred by Penang General Hospital on performing one contact tracing procedure by activity based costing approach. We also aimed to highlight the practical value of this conventional contact tracing procedures in local setting.

Design/Methods: Contact tracing record (including demographic profile of contacts and outcome of contact tracing procedure) from March 2010 until February 2011 was obtained from TB contact tracing record book, retrospectively. Human resource cost was calculated by multiplying the mean time spent (in minutes) by employees doing specific activity to their per-minute salaries. The costs of consumables, Purified Protein Derivative vial and clinical equipment were obtained from the procurement section of the Pharmacy and Radiology Department. The cost of the building was calculated by multiplying the area of space used by the facility with the unit cost of public building department. Straight-line deprecation with a discount rate of 3% was assumed for calculation of equivalent annual costs for building and machine.

Results: Out of 1021 contact tracing procedures, TST was positive (≥10mm) in 38 suspects. However, chemoprophylaxis was started in none. Yield of contact tracing (active tuberculosis) was as low as 0.5%. Total unit cost of chest X-ray and TST was MYR 9.37 (2.94 USD) & MYR 11.80 (USD 3.70), respectively. Total cost incurred on single contact tracing procedure was MYR 21.17 (USD 6.64).

Conclusion: Our findings suggested that yield of contact tracing was very low which might be attributed to inappropriate prioritization process. TST may be replaced with more accurate and specific methods (interferon gamma release assay) in highly prioritized contacts or TST positive contacts should be administered 6H therapy (provided that chest radiography excludes TB) in accordance with standard protocols. Unit cost of contact tracing can be significantly reduced if radiological examination is done only in TST or IRGA positive contacts.

Key words: Tuberculosis, Tuberculin Skin Test, X-ray, activity based costing, contact tracing, Penang General Hospital.

Introduction

Tuberculosis (TB) is a global health tragedy with an annual incidence rate of 9 million cases, worldwide. It is the largest single infectious cause of mortality among young individuals and adults in the world, accounting for approximately two million deaths every year [1]. Similar to other developing countries, TB is still a public health problem in Malaysia despite preventive and control measures taken. The incidence rate of TB in Malaysia has been at around 85 to 82 per 100,000 populations in the last five years. However, the absolute number of new cases has been increasing from about 15,000 new cases in 2002 up to 20000 in 2011 [2]. Pertaining to its highly contagious nature, inadequate investigation of contacts of index case might be one of reasons for its re-emergence.

Fundamental objectives of TB control are to detect disease as early as possible and to make sure that those diagnosed complete their treatment and get cured. In mid 1990s, Directly Observed Treatment Short course (DOTS) strategy was adopted as basis of tuberculosis control [3]. Estimates suggest that the introduction of DOTS could halve the current potential national economic loss from TB [1].

In this era of economic decline, health care managers need to use the most cost effective tracing and treatment measures to halt the progression of

the disease. Contact tracing is the process of identifying the relevant contacts of a person with an infectious disease (index patient) and ensuring that they are aware of their exposure [4]. The World Health Organization (WHO), the International Union Against Tuberculosis and Lung Disease (IUATLD) and the International Standards for Tuberculosis Care (ISTC) recommend as a minimum: a)-screening household and close contacts of smear positive pulmonary tuberculosis cases to detect new TB cases; and b)-for children under five years of age and for all people with HIV without symptoms suggestive of TB, providing isoniazid preventive therapy (IPT) [5-7].

The scope of contact tracing differs in different settings. Tuberculin Skin Testing (TST) and chest X-ray are the most commonly employed contact tracing procedures [4, 8, 9], however TST is comparatively less cost effective than radiological examination [10]. Guidelines [8, 9, 11] on contact tracing recommends TST to all HIV negative household and/ or close contacts of infectious TB cases who are five years of age and above and who have had active TB excluded. Furthermore, guidelines recommend administering Isoniazid (H) preventive therapy in contacts with positive TST. A study from Germany has showed that chemoprevention by Isoniazid is cost-effective approach for reducing the burden of tuberculosis in recently converted young and middle-aged adults [12].

The basic underlying condition of any efficient allocation of resources is the knowledge of cost of illness. Without analyzing costs it is impossible to contemplate or improve the efficiency of disease control projects. In particular, the ongoing reform and decentralization processes in the health care systems of developing countries require precise cost information [13]. Mostly, health care organizations use cost accounting to estimate unit cost of their services that could help to plan a realistic budget and price for the service [14]. Conventional costing systems utilize a single, volume-based cost driver. In most cases this type of costing system allocates the overhead costs to products on the basis of their relative usage of direct labor. This method, has therefore, failed to cope with the challenges of rapidly evolving process and product technologies. It has been well established fact that conventional accounting method overestimates high volume products and underestimates low volume products. This gives an incorrect relationship between production and costs [15].

To date, most of hospital managers rely on information from conventional accounting system that was designed when competition was local rather than global and when pace and quality of item or service was less decisive for success [16]. However, many companies have found a better cost accounting method named as activity based costing (ABC) [17]. ABC approach allows an organization to utilize its resources in best possible way by providing insights into production process for delivering products or services to their consumers [18, 19]. In an activity based accounting system, cost of product or service is the sum of the costs of all the activities required to produce or deliver the service [20]. Accuracy of reported cost is directly proportional to number of activities studied and so does the cost of executing the study [20].

We conducted or study at Respiratory Clinic of Penang General Hospital (PGH), Penang, Malaysia to determine cost of single contact investigation including chest X-ray and TST. We also aimed to compare practices and results of contact tracing in current setting with some of the established protocols. Existing literature suggests that data on the costs and practicality of contact tracing of TB associates is either scarce or unavailable. We expect that findings of our study would have significant impact on principles and practices of contact tracing in local setting and may help other NTPs to review their procedures with similar statistics.

Methods

Setting and study duration

The study was conducted at Respiratory Clinic of PGH. Respiratory Clinic of PGH has a designed Directly Observed Treatment Short Course (DOTS) facility and staff responsible for treatment of registered TB patients. DOTS staff is also responsible for contact investigation of TB associates. Contact tracing records of TB associates were explored from contact tracing log book from 1st March 2010 to 28th February 2011 to explain cost and practicality of procedure in local setting.

Radiology Department of PGH has a designated facility and staff for chest X-ray (labeled as

room 2). Material consisted of 31431 radiological examinations performed during the period of January 1st to December 31st, 2010.

Total costs for chest X-ray included: human resource cost, capital cost, consumable cost and overhead cost. Cost components for TST included human resource and consumable costs.

Contact tracing procedure at study site

Contact tracing procedure at Respiratory Clinic of PGH starts with the notification of index case. After notification, index case details (notification form) are sent to District Health Center (DHC). Health Inspector at DHC visits and conducts an interview of the index case within a week of notification. In case, index case is not available at home, proxy interviews are conducted. After interviewing, details of contacts (including household contacts, friends, colleagues, class fellows) are recorded on specific form. All the listed contacts are asked (either by face to face communication or by telephone) to visit Respiratory Clinic at PGH for their screening for active or latent TB. After finishing interview, one copy of finalized list of contacts is given to responsible staff at Respiratory Clinic of PGH. Once the contact arrives at Respiratory Clinic, DOTS staff responsible for contact tracing performs TST. At the moment contact is counseled to re-visit DOTS center between next 48-72 hours (on third day). At the same time contact is advised to undergo radiological examination (chest X-ray) at Radiology Department of PGH. Developed X-ray film is dispatched to Respiratory Department (attendant 2; see table 2). Medical doctor at Respiratory Department examines the chest X-ray and informs staff nurse at DOTS clinic, if X-ray findings are suggestive of TB. In such case, contact is further investigated using TB specific laboratory tests.

It is the responsibility of Respiratory Clinic staff to inform health inspector if any contact fail to report on the expected dates. In such case, health inspector re communicates with the missing contacts and convince them for contact tracing.

Human resource cost

An interview with key DOTS and radiology personnel was conducted to identify principal activities for TST and chest X-ray. This was followed by determination of the time taken to complete each activity by using a stop watch [21]. The duration was captured 15 times each for alternate three days and summarized as the mean, median, the 25th and 75th quartiles for each activity [21]. The personnel time for each employees involved was valued according to the pay scale of the Federal Civil Services Officers under the System of Remuneration Malaysia [22]. Prior to the valuation, these salaries were converted into the salary per minute (MYR/min) by assuming a daily working time of 8 hours and a monthly working time of 20 days. Cost of each employee per single activity was obtained by multiplying the mean time (minutes) spent by that employee doing a specific activity by his/her salary per minute (MYR/min). Finally, the total manpower cost incurred per service was the sum of human resource costs of all activities involved producing the service.

Moreover, human resource idle time cost for chest X-ray was calculated by multiplying mean idle time between two consecutive activities of each employee with their salary per minute. Idle time cost for medical doctor was not calculated as he/she shared other responsibilities at the Respiratory Clinic of PGH. Similarly, idle time cost for TST was not calculated because staff performing this activity was also sharing other activities at DOTS center. Idle time cost was not included in final cost.

Capital costs

For unit cost calculation of chest X-ray, the costs of equipments were obtained from the procurement section of Radiology Department. The costs of the building were calculated by multiplying the area size for the service with the unit cost of public building (MYR 85/ft^2). Area size of the chest X-ray facility was also provided by the public building department of Penang General Hospital. The useful life was assumed to be five years for clinical equipment and 30 years for building [23]. Moreover, straight-line deprecation with a discount rate of 3% was assumed. At the end of the asset's useful life, the resale value was considered to be 10% of the initial costs [24]. The equivalent annual cost for each was calculated based on the following equations:

Resale value = Asset cost × 0.1
Present value = Resale value × Discount rate

Net present value of the asset cost = Asset cost – Present value

Equivalent annual cost = Net present value of the asset cost / [Annuity factor]

The unit asset cost was obtained by dividing equivalent annual cost of each asset by the total number of X-ray films in the year 2010.

Cost of building and machines was not calculated for TST as DOTS facility was used for certain other TB related activities.

Consumable costs

Consumables for chest X-ray included X-ray film, fixer and developer reagents and envelop for developed film. The quantity and cost of each X-ray film and envelop was obtained from the procurement section of radiology department. Total cost of fixer and developer reagent per service was obtained by dividing total cost of reagents in one year divided by number of tests in year 2010.

Tuberculin PPD RT 23 SST (1.5mL vial) and 1cc syringe were the only consumable for TST. Tuberculin PPD RT 23 SST vial (1.5mL) is recommended for use in 10 individuals but based on number of contacts visiting DOTS during its labeled stability (24 hours), it was used in an average of eight contacts.

Electricity costs

For unit cost calculation of chest X-ray, annual electric power consumption (kW/h) for X-ray machine, day light developer machine and tubes was calculated separately and then multiplied by unit price of one kW/h (MYR 0.312 /kWh) [25] to get annual electric cost for each electrical appliance. Annual electric cost for each appliance was divided by number of X-rays done in 2010 to get cost per X-ray.

Electricity cost was not calculated for TST as DOTS facility was used for certain other TB management related activities.

Data analysis

Socio-demographic and clinical profile of the study participants was presented in frequency and percentage. Staff activity was recorded in minutes. All costs were reported in MYR followed by conversion to US Dollar at an exchange rate of USD1=MYR3.19.

Results

Table 1. Socio-demographic and clinical characteristics of TB contacts

Socio-demographic characteristics	N (%)
Total sample (TST and X-ray)	1021(100)
Sputum positive Index	239
Sputum negative and Extra pulmonary TB index	85
Gender	
Male	435 (42.6)
Female	586 (57.4)
Ethnicity	
Malay	407 (39.9)
Chinese	494 (48.4)
Tamil	53 (5.2)
Others	67 (6.5)
TST reading	
<10mm	547 (53.6)
>10mm	26 (2.5)
=10mm	12 (1.2)
No records	436 (42.7)
Chest X-ray findings suggestive of TB	No record available
Isoniazid started (latent TB infection)	0 (0)
Notified as Confirmed case of TB (active case detection)	5 (0.5)

Human resource costs

Total human resources cost for single contact tracing procedure was MYR 7.43 excluding idle time cost (table 2). Six distinct activities to produce an X-ray film were identified which include receiving and allocating specific number to patient (attendant 1), registering patient in log book (clerk), preparing & exposing patient to X-rays and developing film in day light machine (radiographer 1), labeling film envelop and validating/sorting films to meet standard criteria (radiographer 2), dispatching films to respective wards/clinics (attendant 2) and screening chest X-ray by medical doctor. Radiographer 1 and 2 were the designated staff for chest X-ray, while clerk, attendant1, attendant 2 and doctor were the shared human resources.

For TST, a staff nurse at Respiratory Clinic of PGH performed following duties; a) counseling patient about advantages of TST, recording individual details in contact card and contact tracing log book, b) sub-cutaneous injection of Purified Protein

Table 2. Human resource (HR) cost per chest X-ray film and Tuberculin Skin Testing

Staff	Activities	Mean time (minutes)	Median time (25th,75th) (minutes)	Salary per minute (MYR)	Cost per unit (MYR)	Percentage from total HR cost	Idle time (seconds)	Idle time cost (MYR)
Chest X-ray								
Attendant 1	Receiving and allocating number	1.15	0.90 (0.89,1.3)	0.141	0.162	3.1	8	0.018
Clerk	Patient registration	0.74	0.72 (0.55,1.0)	0.145	0.107	2.2	5	0.012
Radiographer 1	Preparing and exposing patient	3.43	3.0 (2.3,4.6)	0.211	0.723	13.9	12	0.042
Radiographer 2	Labeling and validating film	1.79	1.4 (1.2,2.0)	0.169	0.302	5.8	107	0.299
Attendant 2	Dispatching films	1.36	1.3 (1.3,1.4)	0.141	0.190	3.7	6	0.138
Medical Doctor	Chest X-ray Screening	7.9	8.3 (6.3,8.6)	0.470	3.71	71.3	-	-
Human resource cost per chest X-ray					5.20	Idle time cost per chest X -ray		0.51
Tuberculin Skin testing (TST)								
Staff nurse	Recording contact details, injecting PPD, reading and recording result	14.9	14.7 (13.3,16.3)	0.150	2.23	100	-	-
Total Human resource cost for contact tracing (Chest-X ray + TST) = MYR7.43								

Table 3. Equivalent annual cost and unit cost of assets

Asset	Equivalent annual cost (MYR)	No of test done in 2010	Unit cost (MYR)	Percentage
X-ray machine	51208.3	31431	1.629	82.3
Daylight developer	44825.5	140973	0.318	16.1
Building	1010.4	31431	0.032	1.6
Total unit asset cost			**1.979**	**100**

Derivative (PPD), c) counseling patient to report at Respiratory Clinic between 48-72 hours and d) recording TST result in contact card and contact tracing log book.

Capital costs

Capital costs for TST were not calculated as the facility was shared by other activities/services. However capital costs for chest X-ray included cost of X-ray machine (Philips™), cost of daylight developer equipment (Agfa, Compact EOS™) and cost of building (designed facility for chest X-ray labeled as room number 2). Cost (per film) of X-ray equipment was highest (MYR 1.629) followed by daylight developer equipment (MYR 0.318) and building (MYR 0.032). Total capital cost per chest X-ray film was MYR 1.979 (USD 0.62). Table 3 shows equivalent annual costs and unit costs of assets.

Consumable costs

Total consumable cost for single contact tracing procedure was MYR 11.72. Total cost of consumables for chest X-ray was MYR 2.15. Consumables for chest X-ray included developer and fixer reagents (MYR 0.244 per X-ray film), envelop (MYR 0.257 per X-ray film) and X-ray film (MYR 1.652 per X-ray film).

Table 4. Overall cost per one chest X-ray film and Tuberculin Skin Testing

Resources	Cost (MYR)	Cost (USD)*	Percentage (%)
Chest X-ray			
Human resources cost	5.20	1.63	24.6
Capital costs	1.98	0.62	9.4
Consumable costs	2.15	0.68	10.1
Overhead costs	0.04	0.01	0.2
Total cost	**9.37**	**2.94**	**-**
Tuberculin Skin testing (TST)			
Human resources cost	2.23	0.70	10.5
Consumable costs	9.57	3.0	45.2
Total cost	**11.8**	**3.70**	**-**
Total cost per one contact tracing (Chest-X ray + TST)	21.17	**6.64**	**100**

**1 USD = 3.19 MYR*
(Available from http://www.xe.com/ucc/convert/?Amount=1&From=USD&To=MYR)

Total cost of consumables for TST was MYR 9.57. Consumables of chest X-ray included Tuberculin PPD RT 23 SST (MYR 9.32 per TST) and 1cc syringe (MYR 0.25 per TST). Cost of 1.5 mL vial of Tuberculin PPD RT 23 SST (sufficient for 10 applications) was MYR 74.58 however; unit cost was calculated based on its use in an average of eight contacts per day (24 hours).

Overhead costs (electricity)

Overhead costs for TST were not calculated as the facility was shared by other activities/services. Electricity cost (per film) for X-ray equipment, daylight developer equipment and tubes was MYR 0.002, MYR 0.031 and MYR 0.01, respectively. Total electricity cost per chest X-ray film was MYR 0.043.

Total cost per contact tracing procedure

Total cost for single contact tracing procedure (chest X-ray and TST) was MYR 21.17 (table 4).

Discussion

Prevention of TB infection in healthy individuals is one of the major targets set by World Health Organization (WHO). In most of developed and developing countries, associated of newly diagnosed TB patients should be investigated for active and latent TB infection [26]. However, competing demands restrict the resources that can be allocated to contact investigation of TB associates. Therefore, TB health care manages must decide which contact investigation should be assigned high priority [8]. The criteria to prioritize contacts as high, moderate and low is listed in various guidelines [8, 9, 27], however Malaysian guidelines [28] are silent on this aspect. Our study findings have indicated that prioritization of the contacts by health inspector was done in an arbitrary way without following written procedures which could in fact lead to wastage of valuable resources. Center of disease control and prevention in America has described detailed criteria to prioritize contacts [8]. They have also suggested that prioritization of contacts has favorable impact on efficiency of contact investigation procedure. Looking at the current medical records of index cases [29, 30], determining the infectious period [31-33] and interviewing the patients [33, 34] are components of identifying and prioritization the contacts. Proxy interviews and field investigation [35, 36] of the place are sometimes beneficial. Similarly, anatomical site of disease, results of sputum bacteriology, radiographic findings, age and sociability of index cases are some key indicators which facilitate the decision to initiate contact investigation among contacts. Competency of contact investigation staff is a key to the success of whole process.

Different NTPs employ varying contact tracing procedures depending upon the availability of resources; however TST and chest X-ray are most commonly employed investigations [3, 8, 26, 27]. Most of the countries including United States and United Kingdom limit their contact investigation to high and medium priority contacts which are classi-

fied based on available guidelines [8, 27]. Once the contacts are tagged as high and medium priority, investigation including TST and chest X-ray should be initiated as soon as possible. As per our study findings, health inspectors usually communicated the contacts to visit DOTS center within two weeks irrespective of priority assigned to them. Contrary to this, United States guidelines stress to conduct the test with in seven and fourteen days for high and medium priority contacts, respectively. However a window period of 8-10 weeks is recommended for previously sensitized individuals [8].

Six months of Isoniazid (6H) preventive therapy is recommended in contacts with positive TST results provided that chest radiograph excludes the evidence of TB [3, 8]. Different guidelines suggest different cut off points for a positive TST [3, 9]. WHO and National Institute for Health and Clinical Excellence follow a cut-off point of ≥10mm for positive TST [3, 27]; however, United States guidelines follow a cut-off point of ≥5mm to initiate 6H [8]. According to our study findings, TST was ≥10mm in 38 subjects; however 6H was started in none. This seems to be a clear deviation from the standard protocols. Guidelines on contact tracing from Pacific Island countries suggest not administering TST to contacts, unless the NTP can offer 6H therapy to TST positive contacts and monitor this treatment [9]. United States guidelines on contact tracing suggest employing chest X-ray only when TST is positive. Taking this recommendation into account, this strategy could perhaps, save valuable resources especially in our setting. Contrary to this, other guidelines [9, 27] including United Kingdom guidelines [11] suggest investigating through both TST and chest X-ray. United Kingdom National Institute for Health and Clinical Excellence guidelines further advice interferon-gamma test if TST is positive [27].

It has long been known, however, that the TST is far from ideal, suffering from low sensitivity, low specificity (particularly from significant cross-reactivity to bacille Calmette-Guérin [BCG]) and numerous operational drawbacks. QuantiFERON-TB Gold (QFT-G; Cellestis, Carnegy, Australia) and T-SPOT.TB (Oxford Immunotec, Oxford, UK) are most recent advances for detection of latent TB infection [37, 38]. Both of these test are included in the United Kingdom guidelines, recommending a two-stage strategy of TST testing followed by an Interferon gamma release assay (IRGA) to confirm a positive TST result, although there are no studies that have demonstrated the validity of this approach [39]. Recent United States guidelines that were issued by the Centers for Disease Control and Prevention (CDC) recommend that QFT-G may be used in all circumstances in which the tuberculin skin test (TST) is currently used [40].

A recent meta-analysis [41] has shown that yield of TB contact tracing (active case) in low and middle income countries is 6.5% (aged >15 years). Our findings show an active case detection of 0.5% (active tuberculosis) which is quite low. This huge difference might be associated with the different criteria to prioritize contacts for investigation. However, future studies are required to find the reasons for such a huge gap.

Based on our findings and recommendations by various guidelines, Malaysian protocols used to investigate contacts of TB patients need through revision. TST should either entirely be discontinued or TST positive individuals should be given chemoprophylaxis using 6H or Isoniazid (H) and Rifampicin (R) for 3 months (3HR). The American Thoracic Society is now recommending a regimen of Rifampin and Pyrazinamide (Z) for two months (2RZ) [11]. Opting 3HR or 2RZ preventive therapies could decrease the chances of non-compliance. Authors would also suggest that contact investigation should only be limited to individuals classified as high and medium priority. This measure could perhaps lead to better yield of contact tracing.

One of major strength of our study is that we have employed ABC approach to estimate the cost of single contact investigation. ABC has been successfully implemented in various manufacturing and service organizations however; there have been only few reports on implementation of ABC in health care [42]. To date no study has reported the cost of contact tracing using ABC. Our findings on cost of contact tracing have strong potential to help Malaysian health care managers to take corrective actions for process improvement. For example our findings have shown that radiographer 2 remained idle for 1.78 minutes (107 seconds) which was almost equal to his activity time (1.79 minutes). This clearly suggests that he (radiographer 2) can share another similar activity in the Radiology Depar-

tment, thus saving human resource cost. Similarly, our findings would allow health care managers to evaluate the resources utilized versus benefits achieved. Our findings would also allow policymakers in revising their decision making tree to investigate a TB patient associate keeping in mind the probable unit cost of the investigation.

Conclusion

Our findings suggested that yield of contact tracing was very low which might be attributed to inappropriate prioritization process. Our findings also indicated that chemoprophylaxis was not initiated in TST positive contacts. Therefore, either TST may be replaced with more accurate and specific methods (IRGA) in highly prioritized contacts or TST positive contacts must be administered 6H therapy in accordance with standard protocols. Unit cost of contact tracing can be significantly reduced if radiological examination is done only in TST or IRGA positive contacts.

Study Limitation

While calculating the unit cost of contact investigation, cost of health inspector was excluded as it was not possible for him to recall time and resources (telephone calls, personal visits) spent on each contact.

Ethical approval

Ethical approval was taken from Ministry of Health, Malaysia (ref. dim. KKM/NIHSEC/08/08/04P10-69).

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Impact on Practice

We have provided health care managers and policymakers a base line data on the outcome of their current contact tracing practices.

Our findings could help health care managers to revise forthcoming guidelines on contact tracing.

Our findings would draw an attention to competent authorities in their decision to replace TST with more specific and sensitive tests in highly prioritized contacts or continue it with compulsory chemoprophylaxis in contacts with positive results.

Detailed unit cost calculation of contact tracing will allow health care manager in their decisions for process improvement.

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Turkish reliability and validity of the Index Learning Styles instrument

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Abstract

Background and aim: Differences in the learning style preferences may affect individual academic achievement. Developed by Felder and Solaman the Index of Learning Styles (ILS) instrument divides learning preferences into four categories.

This study aimed to check the reliability and validity of the Turkish ILS instrument.

Methods: ILS instrument and the Kolb's Learning Styles Inventory were applied to 113 students studying at Atatürk University Medical Faculty class 1 during the 2010-2011 period.

Internal reliability coefficient and test-retest calculations were done to check for reliability. Correlations between the reflective observation, concrete experience, abstract conceptualization, and active experience domains of the Kolb inventory and reflective, sensing, intuitive, and active domains of the ILS were calculated to check for concurrent validity. Also factor analysis was performed.

Results: Fifty eight (51.3%) girls and 55 (48.7%) boys participated in the study (total n=113). Predominant preferences were reflective, sensing, visual, and sequential learning styles.

Checking for internal reliability, Cronbach alpha values of the different domains were ranging from 0.289 to 0.631. There were significant correlations in all domains in the test-retest measurements (Pearson r between 0.309 and 0.563; $p< 0.001$).

There were significant correlations between the reflective observation, abstract conceptualization, and active experience domains of the Kolb inventory and reflective, intuitive, and active domains of the ILS inventory respectively (Pearson r and p 0.311 and 0.001; 0.213 and 0.026; 0.307 and 0.001 respectively). In the factor analysis the first four domains produced most important part of the variation.

Conclusion: We conclude that the ILS may be useful in evaluating learning style preferences of medical students. Since there may be significant differences between students, there should be also appropriate diversity in the instructional methods during teaching activities.

Key words: Learning styles, learning styles inventory, Index learning Styles, reliability, validity.

Background and aim

There are considerable differences among learning preferences of students. Some individuals prefer to learn by reading, while others learn better with group discussions, and some by doing. While some people adapt a more deeper learning approach, there are others preferring surface learning approaches[1]. It is theorized that teaching effectiveness and efficiency are optimized when the course design and content closely match students' learning preferences[2]. For this reason, it is extremely important that the individual knows his/her learning style and the teacher takes this into consideration in order to achieve a successful training. It is expected that the preferred learning styles would affect also the performance. Hence, it is suggested to collect data on the preferred learning styles of students and give appropriate counseling[3].

Many tools have been developed to test the learning styles of students. Among the commonly used ones are the Kolb Inventory, VARK, Grasha Reichmann, and Vermunt Learning Styles Inventories[4]. Developed by Felder and Soloman ''Index of learning Styles'' (ILS), divides learning preferences into four areas each with two sub-categories: detection (sensing/intuitive), input (visual/verbal), processing (active/reflective), and comprehension (sequential/global)[5]. The accuracy of all measurements should be guaranteed by checking reliability and validity[6]. This study was designed to adapt Felder and Soloman's Index of Learning Styles instrument into Turkish and assess its validity and reliability among medical students.

Methods

The Felder Index of Learning Styles instrument was translated into Turkish before[7]. ILS and the Kolb Learning Styles Inventory, which were adapted into Turkish before, were applied twice with two-weeks interval to 113 students studying in class 1 at Atatürk University Medical Faculty during 2010-2011. Application of the inventory was on a voluntary basis with prior oral information about the aim and objectives of the study. The study was approved by the local ethics committee of Ataturk University Medical Faculty.

The ILS consists of 44 two-part ('a' and 'b') items, designed to provide scores on the four hypothesized bipolar scales. Each item is treated as contributing to only one of the four scales. There are 11 items for each of the four scales. Total scale scores are computed by summing the scores on the 'a' parts of relevant questions/items and subtracting the sum of the relevant 'b' parts (or vice versa if the 'b' total is greater than the 'a' total). As there are an odd number of items for each scale, a preference will emerge if the respondent completes all items as directed[3, 8] (Kolb Learning Style Inventory. University of Colorado, Center for Astrophysics and Space Astronomy; http://casa.colorado.edu/~dduncan/teachingseminar/KolbLearningStyleInventoryInfo.pdf).

To examine the reliability of the scale, internal reliability coefficients in the four dimensions (Cronbach alpha) and correlations between test - retest reliability were calculated.

The Kolb Learning Style Inventory was used in order to check for concurrent validity. Kolb Learning Cycle was first introduced by David Kolb in 1984 and is based on the principle of the learning cycle which all individuals use to acquire knowledge[9]. It is a simple self-description test, based on experiential learning theory, which is designed to measure the strengths and weaknesses of a learner. Experiential learning is conceived as a four stage cycle: (1) immediate concrete experience is the basis for (2) observation and reflection; (3) these observations are assimilated into a "theory" from which new implications for action can be deduced (4) these implications or hypotheses then serve as guides in acting to create new experiences[8]. Correlations between reflective observation, concrete experience, abstract conceptualization and active experience dimensions of the Kolb inventory and respective dimensions of ILS, namely reflective, sensing, intuitive, and active were checked and factor analysis was performed.

Results

From the 113 participants 58 (51.3%) were females and 55 (48.7%) were males. Mean ± standard deviations of the active/reflective, sensing/intuitive, visual/verbal, and sequential/global domains were -1.2 ± 4.8; 3.6 ± 4.0; 4.5 ± 4.7; and 1.5 ± 3.6 respectively. After recoding and classifying the different learning preferences according to the ILS, we found the following results: in terms of active-reflective balance, 32 participants (28.4%) showed moderate/strong reflective preferences. From sensing-intuitive perspective 58 participants (51.3%) had moderate /strong preference for sensing. In terms of visual-verbal balance, 70 participants (62.0%) had moderate/strong visual preferences. Lastly, 33 participants (29.2%) showed moderate/strong sequential preferences at the sequential-global domain (Table 1).

Although girls were more reflective, sensing, visual, and sequential learners, there was no significant difference between the learning styles of girls and boys ($p>0.05$).

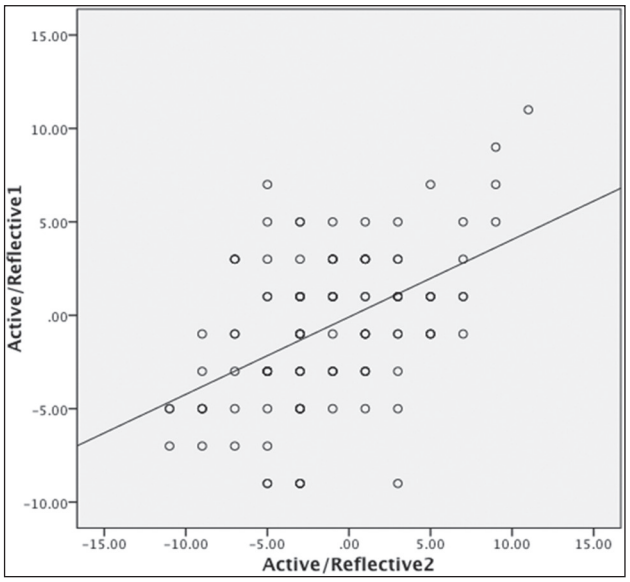
With regard to internal reliability, the Cronbach alpha values for the Active/Reflective, Sensing/Intuitive, Visual/Verbal and Sequential/Global domains were calculated as 0.631, 0.497, 0.667, and 0.289 respectively. In terms of test retest, there was a significant correlation in the Active/Reflective, Sensing/Intuitive, Visual/ Verbal, and Sequential/Global domains (Pearson r; p, 0.482; <0.001, 0.508; <0.001, 0.563; <0.001, and 0.309; 0.001 respectively) (Graph 1).

There was a significant correlation between the Reflective Observation, Abstract Conceptualization, and Active Experience dimensions of the Kolb Inventory and Reflective, Intuitive, and Active dimensions of the ILS respectively (Pearson's r; p, 0.311; 0.001, 0.213; 0.026, and 0.307; 0.001 respectively). On the other hand, there was no significant correlation between Concrete Experience domain of the Kolb Inventory and Sensing domain of the ILS (Pearson r=0.113, P=0.237).

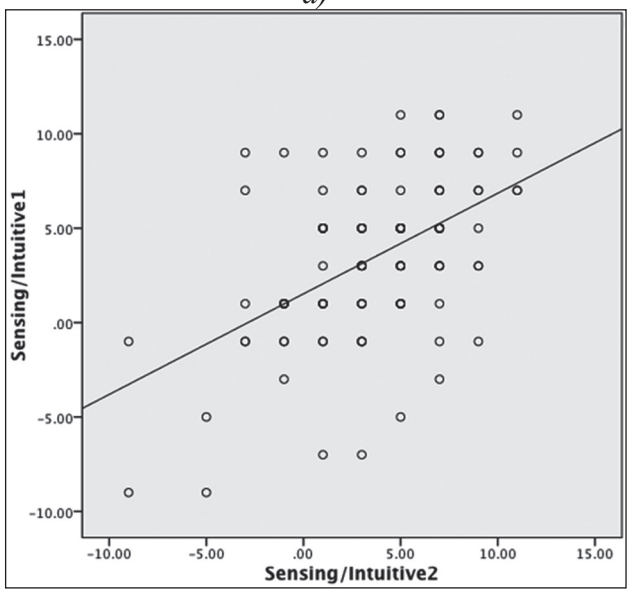
Using factors with eigenvalues of greater than one, 17 domains explained 69.3% of the variation in the factor analysis. Examining the scree plot we found that the most important effect was produced by the first four dimensions (26.7% of the total variance); after the 5th dimension contribution of the factors was decreasing (Graph 2).

Table 1. Learning preferences of medical students according to the ILS inventory

	n	%
Active/Reflective		
Active low preference	28	24.8
Active moderate preference	12	10.6
Active strong preference	4	3.5
Reflective low preference	37	32.7
Reflective moderate preference	23	20.4
Reflective strong preference	9	8
Sensing/Intuitive		
Sensing low preference	36	31.9
Sensing moderate preference	46	40.7
Sensing strong preference	12	10.6
Intuitive low preference	15	13.3
Intuitive moderate preference	2	1.8
Intuitive strong preference	2	1.8
Visual/Verbal		
Visual low preference	24	21.2
Visual moderate preference	42	37.2
Visual strong preference	28	24.8
Verbal low preference	11	9.7
Verbal moderate preference	6	5.3
Verbal strong preference	2	1.8
Sequential/Global		
Sequential low preference	45	39.8
Sequential moderate preference	30	26.5
Sequential strong preference	3	2.7
Global low preference	26	23
Global moderate preference	8	7.1
Global strong preference	1	0.9



a)



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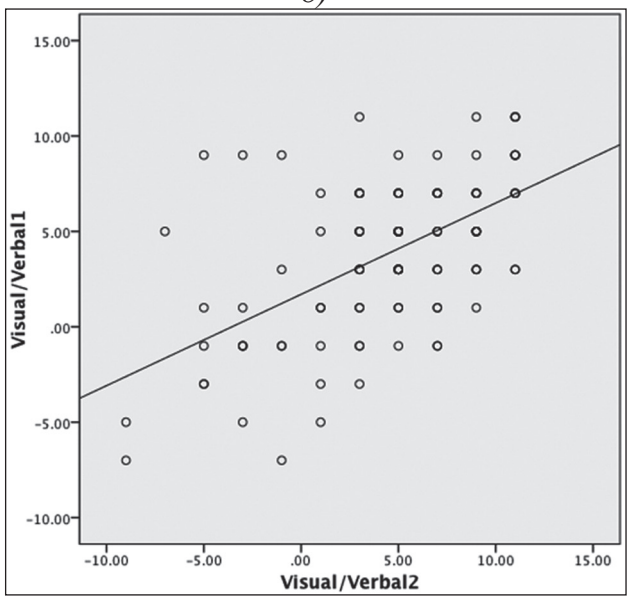
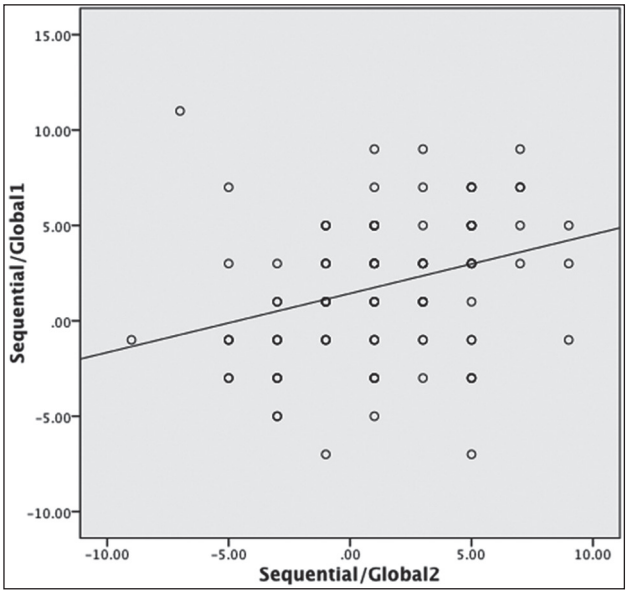


c)

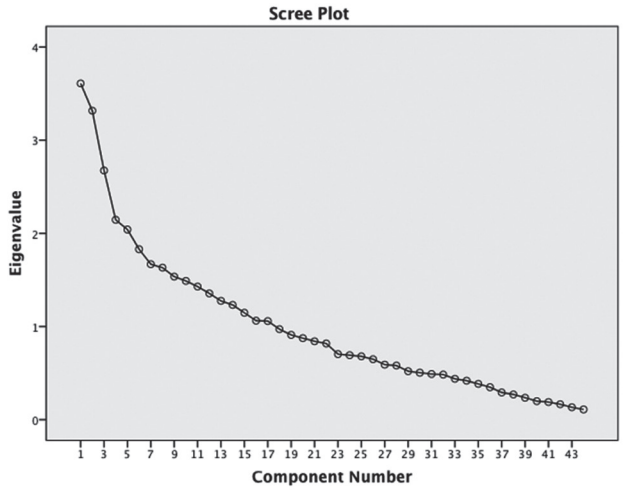
Table 2. Test-retest correlations of different dimensions of ILS found in different studies

Time difference	Active/ Reflective	Sensing/ Intuitive	Visual/ Verbal	Sequential/ Global	N	Reference
2 week	0.482	0.508	0.563	0.309	113	Our study
4 week	0.804	0.787	0.870	0.725	46	Seery et al. [17]
7 month	0.73	0.78	0.68	0.60	24	Livesay et al. [18]
8 month	0.683	0.678	0.511	0.505	124	Zywno [16]



d)

Graph 1. Test – Retest correlations of different dimensions of ILS



Graph 2. Scree plot showing the contribution of different components in explaining the total variances

Discussion

Turkish version of the ILS was found reliable and valid among medical students with regard to internal consistency of items, test-retest correlations and its correlation with the well-known Kolb inventory.

Most of the learners are expected to have preferences in active, sensing, visual, and sequential learning styles 2, 3, 5, 10, 11. We attributed the seemingly difference of our participants' learning styles from the literature to the educational system in Turkey. From primary to high school, the current Turkish educational system is mainly based on didactic lecturing with less place for active experimentation. This may be the explanation why our sample had more reflective learning preferences. Supporting this view, one previous attempt where we applied the ILS to English section medical students (which have more active learning backgrounds) showed 16% active vs. 13% reflective preferences Learning Styles of Our Students (Atatürk University Medical Faculty, Department of Family Medicine; http://aile.atauni.edu.tr/duyurular/2011_1/index.html).

Culturally, we would expect Turkish females to be more reflective. Also using the Kolb Inventory, Australian female students were shown to have higher reflective preferences[12]. However, as to our results although both males and females had reflective preferences, there was no difference with regard to sex.

Min.-Max. Cronbach alpha values for the Active-Reflective, Sensing-Intuitive, Visual-Verbal, Sequential-Global dimensions were previously calculated as 0.48-077, 0.53-0.84, 0.52-0.86, and 0.41-0.81 respectively[3, 13-15]. It is important to mention that most studies revealed Cronbach alpha values around 0.5. Sequential-Global domain has received the lowest internal reliability in all previous studies. Still it needs to be explained why the Turkish version has a value of around 0.3. Also Ku and Shen found the sequential-global scale being the lowest

dimension in the reliability measuring. They identified four problematic items on the sequential-global scale[14]. This domain of the instrument probably needs revision by the original authors.

Felder and Spurlin found test-retest correlation coefficients for all four scales of the ILS between 0.7 and 0.9 for an interval of four weeks between test administrations [5]. Zywno's study on the other hand, had test-retest correlations ranging from 0.51 to 0.68 [16]. When determining test-retest reliability, the interval between test administrations should be large enough so that subjects cannot remember their responses from one administration to the next, but not so large that the quantity being assessed might change to a significant extent in the natural course of events. Intervals from four weeks to 8 months were used in previous studies. Still the duration of two weeks used in our study is long enough to prevent memorizing past answers. Previous test-retest correlations ranged from 0.5 to 0.9 (Table 2).

Although they can't be matched 100%, there are similarities between the ILS and Kolb's model. The active/reflective dimension can be regarded as analogous to the same dimension on the learning style model of Kolb. Also the sensing/intuitive dimension may have a counterpart in the concrete/abstract dimension of the Kolb model. Although there are similarities in the Kolb and ILS inventories, there is no complete match between the different dimensions. Therefore, we believe it is still important to have agreement in three out of four dimensions.

Zywno found the number of factors extracted with eigenvalues less than 1.0 as 14, accounting for 54.1% of the total variance. Using the "scree plot" test, the number of extracted factors was equal to 5 [16]. The first method (Kaiser criterion) sometimes retains too many factors, while the second (scree test) sometimes retains too few, however, both do quite well under normal conditions, that is, when there are relatively few factors and many cases. David Ku and Chun Shen on the other hand, explained the ILS with eight factors[14].

We conclude that the Index Learning Styles Inventory is a useful tool to evaluate the different learning styles of medical students. Since there may be significant differences between students, a variety of instructional methods should be incorporated during teaching activities.

Authors' contributions

ZA designed the study, helped with analysis, drafted the manuscript, and approved the final version.

HA helped with acquisition of data, data analysis, and final approval of the manuscript.

TS helped with drafting of the manuscript.

ZC helped with drafting of the manuscript.

ZA helped with drafting of the manuscript.

All authors read and approved the final manuscript.

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Comparison of Toxoplasmosis frequency in pregnant women during two years in Qom province (iran)

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Abstract

Background: *Toxoplasma gondii* is an obligate intracellular protozoan parasite occurring with a global distribution in human and animals. The purpose of this work was to evaluate the frequency of toxoplasmosis among pregnant women of Qom province using current serological methods during two years (2009- 2010).

Materials and Methods: In this descriptive cross-sectional study, 200 serum samples from βHCG-positive women were randomly selected, transferred to laboratory, and examined by ELISA and IFA methods. The effects of some factors on incidence of the disease were then statistically evaluated.

Results: According to the results obtained, the prevalence of Anti-*Toxoplasma* was 39.5% and 45.3% for 2009 and 2010, respectively. The results demonstrated that the prevalence rates of *T. gondii* IgG antibody in this population were 34.5% (69/200) and 39.6% (76/200), and the prevalence of *T. gondii* IgM antibody were 5% (10/200) and 5.7% (11/200) in 2009 and 2010, respectively.

Discussion: The prevalence rates of toxoplasmosis were not significantly different in the two years of study. Regarding the results, about half of the women studied were at the risk of infection with *T. gondii*. So, scientific and practical preventive measures should seriously and continuously be applied.

Key words: Toxoplasma, Antibody, Pregnancy, IgM, IgG.

Introduction

Toxoplasmosis is caused by the intracellular parasite *Toxoplasma gondii* that causes a persistent infection in 10-80% of the world's population, depending on geographic location. The infection is caused by consuming contaminated meat or coming into contact with cat feces containing oocysts. *T. gondii* infects a large proportion of the world's population from temperate to tropical areas. Individuals at risk include fetuses, newborns, and immunologically compromised individuals.

If a pregnant woman becomes infected by toxoplasmosis, the parasite may pass through the placenta to the fetus, resulting in congenital toxoplasmosis, which is a cause of mortality and malformation (1, 2).

Therefore, the present study examines the prevalence of *T. gondii* parasitemia in pregnant women using both enzyme-linked immunosorbent assay (ELISA) and indirect fluorescence antibody test (IFA). The results from different studies indicate that various rates of infection have been reported in different parts of Iran and world. Even in one country, the infection rate varied from one place to another (3). There was not any new report available on the prevalence of toxoplasmosis in Qom. Therefore, there is a need to carry out a study on pregnant women in Qom to estimate the burden of problem in this area.

Materials and Methods

In this cross-sectional study, the samples were randomly obtained from women who were referred to the state obstetrics hospital of Qom for βHCG test, and the test result was positive. In the study, the sample sized was 200 cases, and the d value was determined to be 0.04 at a confidence level of 96%, based on the infection prevalence rate of 35%. Blood samples were collected and the sera was separated by centrifugation at 3000 rpm for 5 min and then frozen at -20 °C until use. In

IFA test, antigen prepared from tachyzoites of *T. gondii* RH strain was used. Briefly, tachyzoites of *T. gondii* RH strain were inoculated in peritoneum of Balb/c mice. After four days, tachyzoites were collected by peritoneal washing and centrifuged at 2000 rpm, washed three times with PBS, coated on microscopic slides, and were frozen at -20 °C until use. Sera were diluted serially and *Toxoplasma* antibodies (IgG or IgM) were detected with indirect immunofluorescent antibody method. Titers higher than 1:10 were considered positive. Enzyme-linked immunosorbent assay test was used to screen all the samples for T. *gondii* antibodies according to the manufacturer's guidelines. Testing was carried out in batches. Briefly, 96-well microtiter polystyrene plates were sensitized with sonicated *T. gondii* antigen provided with the kit at a concentration of 100 μg/ml in carbonate buffer (pH 9.5) and blocked with bovine fetal serum to 1% in PBS-Tween 20 (0.01%). The sera were diluted1:100 using the assay diluent supplied with the kit and incubated at 25 °C for 30 min, and rinsed four times with wash buffer. Then, 100 μl of the detecting anti-IgG conjugate or anti-IgM conjugate was added to each well. Plates were incubated humidified at 37 °C for 30 min and were then washed five times before addition of 100 μl of tetramethylbenzidine substrate. The plates were incubated for 10 min at room temperature in the dark, and the colorimetric reaction was stopped by adding 100 μl of stopping reagent per well. The absorbance rates of the samples and controls were determined at 450 nm by ELISA microplate reader. Positive and negative control sera were included in each run. The results were compared with cut off and expressed in IU/ml by quantitative estimation using calibration curve constructed with cutoff and three positive controls (an index value of <0.253 was considered negative for anti-*Toxoplasma* antibody, a value of ≥ 0.253 but ≤ 0.343 is considered equivocal, and a value of > 0.343 is considered positive). Equivocal results were not included in the analyses. Ethical Committee of the University approved this study. Data on age, contact with cat, habit of undercooked meat ingestion or unwashed/unpeeled vegetables, and area of residence were obtained using questionnaires. We used the SPSS 9.0 software for analyzing the data from these experiments. In order to check for statistic difference, chi-square test and Student's *t*-test were adopted. Differences between the two groups were considered significant when p values were < 0.05 (4, 5).

Results

This study was an attempt to define the epidemiology of *T. gondii* infection in 200 pregnant women in Qom. The average of two methods used showed that the prevalence of Anti-*Toxoplasma* was 39.5% and 45.3% for 2009 and 2010, respectively. Among the women studied, 60.5% and 54.7% were seronegative toxoplasma (high risk) in 2009 and 2010, respectively. We did not find statistically significant difference between the disease prevalence rates in the two years of study. Among the 200 serum samples analyzed, 79 and 87 samples were found to be positive for anti-*T. gondii* IgG and IgM, corresponding to an overall prevalence of 39.5% and 45.3% in 2009 and 2010, respectively. The average of the results obtained by the two methods showed that most positive samples in 2009 and 2010 (69 and 76 cases; 34.5% and 39.6%, respectively) were positive for IgG, which indicates chronic infection. Moreover, a lower rate of the positive samples (5% and 5.7% or 10 and 11 cases in 2009 and 2010, respectively) were positive for IgM antibody, which indicates acute infection. The highest infection rate was observed in the age range of 20- 30. Regarding IgG, there is a statistically significant relationship between the age and occurrence of the disease. However, such relationship was not found for IgM. For the population under study, 65% those who had contact with cat were positive for IgG, and 25% were positive for IgM. Moreover, 40% of women who live in cities and 30% of women who live in villages were positive for IgG. The positive IgM rates were 70% and 15% for women living in cities and villages, respectively. Among the women living in rural areas, 70% and 25% of those who use semi-cooked meat and unsterile row vegetables were positive for IgG and IgM, respectively. The results showed that using semi-cooked meat and unsterile row vegetables, having contact with cat, and the residence place have a statistically significant effect on the rate of toxoplasmosis. The symptoms and signs reported

in the patients were fever, jaundice, muscle pain, GI symptoms, minor discomfort, skin blisters and rashes, lymph node swelling, and chilling. With minor differences, the diagnostic value IFA and ELISA were almost equal.

Discussion

Toxoplasma is a globally distributed pathogen of human and animals. Almost 30- 80% of human population carry latent infection of this opportunistic parasite. Since current treatments are not fully effective against the infection, and no *T. gondii* vaccine is available, efforts to reduce toxoplasmosis transmission are crucial to reduce the impact of this disease. Our study showed that in 2009 and 2010, 39.5% (79/200) and 45.3% (87/200) of pregnant women were seropositive for *T. gondii*, respectively. It was observed that in the population studied, the prevalence of *T. gondii* antibody was 34.5 (69/200) and 39.6% (76/200) for IgG and 5% (10/200) and 5.7% (11/200) for IgM in 2009 and 2010, respectively. The highest rate of infection was observed in the age range of 20-30. In a previous study on pregnant women of Qom city in 2003, the two methods of IFA and ELISA were compared in seroepidemiological study of *Toxoplasma* infection. The study was performed on 600 serum samples, it was observed that 257 (42.8%) and 246 (41%) individuals were positive for specific IgG antibody by IgG-ELISA and IgG-IFA methods, respectively. In comparison of the two methods of IFA and ELISA, it was demonstrated that 246 cases (41%) were positive in both methods, and 343 cases (57.2%) were negative in both methods, and only 11 cases (1.8%) were positive in ELISA and negative in IFA evaluation. The prevalence rates reported in previous studies were 84% in Tehran, 54.2% in Kashan, 42.8% in Qom, and 45.5% in Karaj (6-9).

In Europe, the highest rates are observed in the central and southern Europe, while the lowest rates are found in the northern Europe. The infection prevalence rates in other countries were 79%, 30.1%, and 31.7% in Korea, Turkey, and Jordan, respectively (10-12). Although the results obtained in the current study indicate that transmission of *Toxoplasma* infection in Qom is almost similar to other parts of world and Iran, it was observed that a significant percentage of pregnant women did not have any type of acquired immunity against toxoplasmosis. The difference may be attributed to climate condition, nutritional behavior, socioeconomic state, and keeping cats as pets. However, the differences observed among seroprevalence rates in different countries can be the result of assay and sampling methods and also geographic and other temporal factors. The effect of these factors could be evaluated by carrying out studies on the public health level, individual characteristics and habits, geographical and regional conditions, and different methods of serological evaluation of antibodies against *Toxoplasma.* Considering the results obtained, 60.5% and 54.7% of the pregnant women studied were at the risk of *T. gondii* infection in 2009 and 2010, respectively. So, implementation of preventive measures should be considered. The result indicated that pregnant women at the risk of the infection should consult with an obstetrician. In this study, a significant relationship was found between age and the positive result for IgG against *Toxoplasma*. However, such relationship could not be confirmed for IgM. The reason for higher titers of the antibody in higher ages is not clear. A probable explanation could be the increased cumulative exposure to the parasite at higher ages. The rate of toxoplasmosis had a statistically significant relationship with residential place (urban vs. rural), consumption of semi-cooked meat and unwashed raw vegetables, and being in contact with cat. These variables have been introduced as the major risk factors of the disease in other studies carried out in other regions with different personal and public health conditions. In this study, the infection rate was not significantly different in the two years of study. Therefore, carrying out pre-marriage tests and training of the people, especially pregnant women, about the disease, and vigilance in the pregnancy period is necessary. Furthermore, the results showed that ELISA is preferred to IFA in screening of toxoplasma infection, owing to its high sensitivity and specificity, simple application, and lower costs (8, 11, 12).

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Improvement in skin barrier function following long-term treatment with green tea and lotus in healthy adults, a step towards future treatment of atopic dermatitis

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Abstract

Objective: The determination of this in vivo study was to investigate, non-invasively on human subjects, changes in skin barrier function following long-term topical application of green tea and lotus.

Methods: Three groups of 33 male subjects each with 11 subjects were studied during the winter season. One group applied green tea on one side of the face and placebo on other side of the face. Similarly second group applied lotus and third group applied combined treatment of green tea and lotus in a 60 days treatment course. Biometrological measurements of hydration and transepidermal water loss (TEWL) were performed on both sides of the face in each group at baseline and on day 15, 30, 45 and 60.

Results: The statistical interpretations revealed green tea mono treatment is superior compared to placebo as it showed extremely significant improvements in skin epidermal hydration and TEWL through 60 days treatment course (two-tailed P value equals 0.0001 and 0.0090 respectively). Lotus mono treatment showed significant improvement in epidermal hydration while effect was extremely significant on TEWL (two-tailed P value equals 0.0144 and 0.0001). In case of combined treatment, apparent improvement in epidermal hydration was dramatic up to 57.29 % at the completion of study with respect to baseline value but results were statistically non-significant. On the other hand TEWL reduction was not impressive enough as expected though statistically significant reduction compared to placebo has been observed with combined treatment (two-tailed P value equals 0.0329).

Conclusion: Results are promising for all tested formulations hence future studies are necessary to clinically evaluate these preparations in conditions with compromised skin barrier especially against atopic dermatitis.

Key words: Green tea, corneometry, TEWL, multiple emulsions, atopic dermatitis.

Introduction

The largest and the outermost organ of human body, skin, is responsible for regulatory and multiple defensive functions. Stratum corneum (SC), the skin superficial layer lies in epidermis particularly plays important role of skin barrier function (1). TEWL is in-vivo measurement method for testing stratum corneum barrier function of the human skin. The stratum corneum layer of the epidermis is outermost layer of the skin which acts as main barrier. It maintains selective permeability of the substance in and out of the skin. More over glycolipids in the epidermis prevent water loss from the body. On the other hand dermis contains water, ground substance and elastic fibers (2).

The skin barrier fulfills various defensive functions:

a) protection from environmental factors (physical, chemical, biological);
b) antimicrobial protection
c) regulates the transport of water and the exchange of substances with the environment (excretion, secretion, resorption) and
d) protection against oxidative stress.

A precise regulation is needed for the proper implementation of these functions. There are several interrelated mechanisms and signaling systems for the formation and maintenance of the epidermal barrier among that hydration of the stratum corneum

is considered the primary one (3). Disruption of this function results in increased transepidermal water loss or TEWL and is associated with conditions like atopic dermatitis and other chronic skin diseases (4).

A daily moisturizing routine is a vital part of the management of patients with atopic dermatitis and other dry skin conditions. The composition of the moisturizer determines whether the treatment strengthens or deteriorates the skin barrier function, which may have consequences for the outcome of the dermatitis (5). There are currently several products that contain green tea extract on the market. Unfortunately, the concentration of phenols is not standardized in these products; therefore, some products may have little-to-no therapeutic effect, making purchasing them a challenge for consumers. It is generally accepted that five-percent green tea extract or polyphenols in the 90-percent range is an effective concentration (6). Polyphenols that are admired antioxidants in cosmeceuticals, found in green tea are catechins. Epigallocatechin -3- gallate (EGCG) is approximately 59 % of total catechins found in green tea. Green tea also contains gallic acid, kaempferol, myricetin and quercetin (7). Like green tea, lotus whole plant is rich in protein, amino acids, unsaturated fatty acids, alkaloids, minerals and flavonoids which provide extract of this plant with diverse beneficial effects for human health (8).

The progress in biometric techniques, have made it possible to measure changes in skin barrier function non-invasively. The aim of current study was to evaluate the changes in skin barrier function following 60 day treatment with green tea and lotus alone or in combination carried to the skin through novel multiple emulsions.

Methods

Subjects

In this study thirty three healthy subjects (Mean age 25 ± 3.97) recruited in this study, further divided into three groups each group having 11 volunteers. All volunteers completed the study effectively. None of them has pathological condition on the area specified for applying the test products. They were properly informed about the use of products and necessary details about study. Furthermore, they were instructed not to use any skin care products like moisturizers on the test sites 15 days before study and throughout the study period of 8 weeks. Moreover they were asked not to change their dietary habits during the study to nullify the effects of such changes on study results.

Test preparations

Products tested were W/O/W type multiple emulsions, loaded with green tea (GT), lotus (L) or combination of both (GT-L). Compositions of products tested are shown in table 1. Each participant provided with two vessels with 30 g contents, marked right and left, respectively for use daily at bed-

Table 1. Composition of different tested formulations

	Composition (% w/w)			
	Placebo	**GT**	**L**	**GT-L**
Simple emulsion (W/O)				
Paraffin oil	24	24	24	24
Abil® EM 90	4.25	4.25	4.25	4.25
Green Tea extract	---	5	---	2.5
Lotus extract	---	---	5	2.5
Magnesium Sulfate	0.7	0.7	0.7	0.7
Deionized water (Q.S)	100	100	100	100
Multiple emulsions (W/O/W)				
Simple emulsion	80	80	80	80
Brij 58	3.75	3.75	3.75	3.75
Cetomacrogal 100	2.5	2.5	2.5	2.5
HPMC	1.25	1.25	1.25	1.25
Deionized water (Q.S)	100	100	100	100

time on each half of the face. They were instructed about proper application of the products and were reminded regularly about the use of product to ensure 100 % compliance in use of these products.

Study design

This placebo controlled, split-face, monocentric study was conducted to evaluate the effects of antioxidants from green tea and lotus for the improvement in skin epidermal function. This study conducted during the winter months and a single expert investigator ensured the proper conductance of biometrology measurements, considering the experimentation protocols for this skin measurement technique. One cosmetic expert continued throughout the study to minimize person to person variations. Moreover assessor not informed about the contents of tested products to ensure blindness in the study. Tests were carried out on right and left cheeks of healthy adults unknown about contents of the formulations. Assessments for objective, skin profilometry were performed at baseline, on day 15, 30, 45 and 60. Before any measurements, all volunteers had to rest in Cosmetic Lab, under constant environmental conditions of 20±2 °C and 45±5% relative humidity, for at least 30 minutes in accordance with the protocols set for these measurements.

Ethical considerations

This study was approved by the Board of Advance Studies and Research and Ethical Review Committee, The Islamia University of Bahawalpur (No. 942/Acad).The study was conducted in accordance with the ethics principles of the Declaration of Helsinki and was consistent with Good Clinical Practice guidelines. All participants include in this study after written informed consents. They were informed about possible adverse reactions, procedures, protocols and objectives of this study. They reserved the rights to quit study without informing about such reasons.

Instrumental measurements

Biometrology, noninvasive probes have been used in this study for the measurement of skin epidermal function. The Corneometer® (Courage & Khazaka, Germany) used measures electrical capacitance of the skin surface expressed in arbitrary units (a.u) ranging from 0-120 a.u so called corneometric indexes (9). The instrument could assess the epidermal hydration of the stratum corneum to a depth of approximately 0.1 mm. The probe was applied to the skin with a spring loaded standard force of 3.5 N and the results were shown digitally in arbitrary units within 3 seconds of applying the probe. The measurement of trans-epidermal water loss (TEWL) performed by a Tewameter® (Courage & Khazaka, Germany) which is based on the diffusion in an open chamber and is measured as $g/m^2/h$.

Statistical Analysis

The data for measured parameters has been analyzed using statistical Graphpad software. The data has been analyzed using two-tailed, paired Student's t-tests for the statistical analysis and P-values of less than 0.05 were considered statistically significant while $P < 0.01$ considered for extremely significant effects. Percent changes shown in the tables indicate the difference from baseline values at different time intervals.

$$\% \text{ Change} = [(Dx - D0)/D0] \times 100$$

where Dx is the value obtained at the dermatological tests on day 30 and 60 (D30, D60). Experimental error is expressed in the table as the standard error of measurement (SEM).

Results

In this study thirty three participants (mean age 25 ± 3.97) enrolled in this study after their written informed consents. None of the patients reported any discomfort or side effects following long term use of the treatments. The percent changes for the epidermal function parameters following long term treatment with placebo, green tea (GT), lotus (L) and green tea plus lotus (GT-L) along with statistical interpretations, has been shown in table 2, 3 and 4.

Effect of treatments on epidermal hydration

Placebo side of the face in green tea group shown initial fall in epidermal hydration (-0.06 % after 15 days) and slightly improved epidermal hydration after 60 days treatment course (8.06 %). Placebo side of the lotus group shown slight improvement in epidermal hydration after 15 days treatment (6.97 %), however the effect terminated

*Table 2. Changes in measured skin epidermal function after 60 days topical application of a multiple emulsion loaded with 5 % green tea extract. Two tailed significant difference after 60 day treatment course has been mentioned as *P < 0.05 (significant) while ** P < 0.01 (extremely significant).*

	% Changes in epidermal function		
	Placebo	**GT**	**P value**
% Changes in Epidermal hydration	8.06±7.86	31.34±6.60	0.0001**
% Changes in Trans epidermal water loss	-8.69±8.30	-43.17±8.17	0.0090 **

*Table 3. Changes in measured skin epidermal function after 60 days topical application of a multiple emulsion loaded with 5 % lotus extract. Two tailed significant difference after 60 day treatment course has been mentioned as *P < 0.05 (significant) while ** P < 0.01 (extremely significant).*

	% Changes in epidermal function		
	Placebo	**L**	**P value**
% Changes in Epidermal hydration†	2.25±9.60	15.39±11.06	0.0144*
% Changes in Trans epidermal water loss‡	-0.66±14.10	-25.16±10.71	0.0001**

*Table 4. Changes in measured skin epidermal function after 60 days topical application of a multiple emulsion loaded with combination of green tea and lotus (2.5 % lotus and 2.5 % green tea). Two tailed significant difference after 60 day treatment course has been mentioned as *P < 0.05 (significant) while ** P < 0.01 (extremely significant). NS denotes non-significant change.*

	% Changes in epidermal function		
	Placebo	**GT-L**	**P value**
% Changes in Epidermal hydration	4.03±4.40	57.29±20.81	0.6879NS
% Changes in Trans epidermal water loss	4.61±12.23	-34.85±6.43	0.0329*

gradually after 30 days (-1.64 %), 45 days (-2.18 %) and slight regain after 60 days (2.25 %). Placebo side in GT-L treated group has shown sustained results, i.e. 2.46 %, 4.26 %, 4.80 % 4.03 % at 15, 30, 45 and 60 days respectively.

Contrary to placebo treatment effects gradual improvement in epidermal hydration has been observed after treatment with GT, L and GT-L. For example in green tea treated group, green tea treated side of the face has shown gradual improvement in epidermal hydration and effect continued after 15 days treatment (6.50 %) till the end of study (31.34 %). The statistical interpretations revealed green tea mono treatment is superior compared to placebo as it showed extremely significant improvements in skin epidermal hydration through 60 days treatment course (two-tailed P value equals 0.0001).

In lotus treated subjects, effect was towards gradual improvement after each time interval but the intensity of effects was half to that of green tea treated group. The changes were -0.55 % after 15 days, 7.94 % after 30 days, 11.99 % after 45 days and 15.39 % at 60 days. Statistical data demonstrates that lotus treatment showed statistically significant improvement in skin epidermal hydration compared to placebo treatment in a 60 days treatment course (two-tailed P value is less than 0.0144). By conventional criteria, this difference is considered to be statistically significant.

Most prominent epidermal hydration effects were recorded after with treatment GT-L. Effects were intense in magnitude i.e. 15.58 % after 15 days as well as sustained at other time intervals i.e. 53.33 % after 30 days, 50.45 % after 45 days and 57.29 % at the completion of study. But statistically differences are not statistically significant, compared to placebo treatment over 60 days treatment course (two-tailed P value equals 0.6879).

Effect of treatments on trans-epidermal water loss (TEWL)

When we observed placebo side of the face in green tea group, placebo treatment has shown initial decrease in TEWL i.e. -5.30 % after 15 days and effect potentiated after 45 days up to -10.01 %, remained sustained till 60 days i.e. -8.69 %

compared to baseline TEWL value of the participants. Placebo side of the lotus group has shown slight increase in TEWL values to that of baseline value 1.28 % after 15 days, 1.13 % after 30 days and 3.76 % increase after 45 days while a slight reduction after completion of study period -0.66 %. Placebo side in GT-L treated group has shown initial control over TEWL i.e. -6.97 % after 15 days and subsequent termination of effect with the passage of time i.e. -2.58 % after 30 days, -0.37 % after 45 days and an increase in TEWL at the end of 60 days i.e. 4.61 %.

Different to placebo treatment effects some excellent improvement in barrier function has been observed after treatment with green tea i.e. -34.08 % reduction in TEWL after 15days treatment, -37.30 % reduction after 1 month, -40.69 % reduction after 45 days and -43.17 % reduction in TEWL at the end of study period to that of baseline values. The statistical interpretations shown green tea single treatment is superior compared to placebo as it showed extremely significant reduction in trans-epidermal water loss through 60 days treatment course (two-tailed P value equals 0.0090)

Similar to green tea single treatment, lotus treated side of the face has shown marked reduction in TEWL at 15 days measurement i.e. -32.16 %. Unfortunately with the passage of time lotus treatment failed to produce sustained effects, thus reduction in TEWL at 30 days, 45 days and 60 days was -24.20%, -23.61 %, -25.16 % respectively.

However, statistical data demonstrates that lotus treatment showed statistically significant reduction in TEWL compared to placebo treatment in a 60 days treatment course (two-tailed P value is less than 0.0001). By conventional criteria, this difference is considered to be extremely statistically significant.

The effect of combined treatment was impressive enough as we were expecting, rather the effect was gradual over time with respect to baseline value. Changes were in the following order; -10.46 %, -22.43 %, -31.45 %, -34.85 % at 15, 30, 45 and 60 days respectively. Statistical data demonstrates that combined treatment has significant effect on trans-epidermal water loss in a 60 days treatment course, compared to placebo treatment (two-tailed P value equals 0.0329). By conventional criteria, this difference is considered to be statistically significant.

Discussion

ROS balance is maintained through a controlled mechanism by the living tissues and many endogenous antioxidants come into action once these ROS are generated in-vivo. However, when endogenous antioxidants become insufficient or imbalanced in defense against oxidants, exogenous antioxidants may help restore the balance. Antioxidants inhibit the production of ROS by direct scavenging, decrease the amount of oxidants in and around our cells, prevent ROS from reaching their biological targets, limit the propagation of oxidants such as the one that occurs during lipid peroxidation, and thwart oxidative stress thereby preventing the aging phenomenon. In this regard green tea phytoantioxidants role play in dual fashion i.e. first they inhibit the production of ROS and secondly they protect the endogenous antioxidants (10).

The most commonly technique to measure barrier function is measure of transepidermal water loss (TEWL), as it indicates how much water is being lost to environment under damaged barrier (9). Furthermore, an inverse relationship between TEWL and SC hydration is well known. High TEWL values, as a marker of disturbed skin barrier function, are frequently correlated with low hydration of the SC as shown in experimental settings after skin cleansing with soaps and detergents or in diseased skin (11).

Results of our study are consistent with above stated hypothesis. For example in green tea treated group, green tea treated side of the face has shown gradual improvement in epidermal hydration and effect of green tea mono treatment was superior compared to placebo as it showed extremely significant improvements in skin epidermal hydration through 60 days treatment course (two-tailed P value equals 0.0001). On its effects on TEWL -43.17 % reduction in TEWL at the end of study period to that of baseline values has been observed in our study. The statistical interpretations shown green tea single treatment is superior compared to placebo as it showed extremely significant reduction in trans-epidermal water loss through 60 days treatment course (two-tailed P value equals 0.0090). Similarly lotus mono treatment has shown significant differences compared to placebo for its effects on epidermal hydration and TEWL (two-tailed P

value equals 0.0144 and 0.0001) respectively. In case of combined treatment, apparent improvement in epidermal hydration was dramatic up to 57.29 % at the completion of study with respect to baseline value but results were statistically non-significant. On the other hand TEWL reduction was impressive enough as expected though statistically significant reduction compared to placebo has been observed (two-tailed P value equals 0.0329). In our opinion apparent hyper hydrated effect produced by combined treatment has influenced TEWL reduction compared to mono treatments. This phenomenon is also supported by a study stating elevated TEWL levels can be found both in hyperhydrated and in dry skin (12). Irrespective of the above said phenomenon, combined treatments have been proved superior over mono treatments in improving stratum corneum barrier function (13, 14).

The most serious damage to skin barrier is induced by UV radiations and topical application of green could offer protection against detrimental effects of UV on cutaneous immunity. (15). Regular intake of EGCG strengthens the skin's tolerance by increasing MED and thus prevents UV-induced perturbation of epidermal barrier function and skin damage. These results suggest that EGCG is a potent candidate for systemic photoprotection (16).

Atopic dermatitis (AD) is a chronically relapsing inflammatory multifactorial skin disease with genetic background, immune abnormality and environmental factors. Recent studies have identified skin barrier damage as a primary cause of the disease. The skin of patients with AD harbors several defects in epidermal barrier function and the severity of the barrier defect parallels AD severity (17, 18, 19).

In this study we have utilized several quantitative techniques (hydration by Corneometry, TEWL by Tewameter,) to measure changes in skin barrier function. We have found that effects produced by green tea are in accordance with the literature reporting protective effects of various polyphenols against UV-induced photo oxidation, induction of inflammation, oxidative stress, and DNA damage from different stress sources in cell cultures and animals (20).

On the other hand extract of *Nelumbo nucifera* stimulates defense system by modulating several immunological parameters (21). More specifically, a study was conducted to examine the effect of *Nelumbo nucifera* (Gaertn.) leaf (NL) on the AD-like skin lesion induced by repeated epicutaneous application of 2,4-dinitrochlorobenzene (DNCB) on the dorsal skin of NC/Nga mice. The efficacy of NL was judged by histopathological examination, blood IgE level, measurement of transepidermal water loss (TEWL), scratching behavior, and skin severity score. Results suggested that NL may be a useful natural resource for the management of AD (22).

The quantitatively measured values of skin hydration and TEWL following long term treatment with green tea, lotus alone or in combination have led us to conclude that results are promising and future studies are necessary to clinically evaluate these preparations in conditions with compromised skin barrier. Perhaps, these safe and effective preparations could be convenient a lot in the treatment of multifactorial atopic dermatitis disease.

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Urinary tract infections caused by Double-J catheters in the period of pregnancy

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Abstract

Purpose: The aim is to evaluate the urinary tract infections caused by double-J (DJ) catheters used in the treatment of hydronephrosis of pregnancy.

Materials and methods : 42 patients, who admitted due to the symptomatic hydronephrosis in pregnancy and who the ureteral DJ catheter was administered, were evaluated. The patients were evaluated with the complete urinalysis, urine culture, serum urea and creatinine as well as ultrasonography before and after the application of the ureteral DJ catheter. The patients who had the urinary tract infection were excluded from the study before the ureteral DJ catheter was hooked up. Conservative treatment consisted of hydration and analgesics, when they are needed, were performed to all of the patients.

Results: 42 patients with hydronephrosis and who the ureteral DJ catheter was administered were included to the study. It was the first pregnancy of twenty-four patients (57.1%). Hydronephrosis of grade II in 24 renal units and hydronephrosis of grade III in 18 renal units were detected. There were right hydronephrosis in 28 patients, left hydronephrosis in 13 patients and bilateral hydronephrosis in one patient.. There was no the determination of bacterium in urine culture of 30 (71.4%) of 42 patients who the ureteral DJ catheter was performed. There was the determination of bacterium in urine culture of 12 patients (28.6%). It was detected that there were asymptomatic bacteriuria in 5 patients (12%), acute cystitis in 4 patients (9.5%), and acute pyelonephritis in 3 patients (7.1%).

Conclusions: In the treatment of symptomatic pregnancy of hydronephrosis which did not respond to the conservative treatment, urinary tract infections can be seen in different groups from asymptomatic bacteriuria to the acute pyelonephritis after the application of ureteral DJ catheter. Therefore, the patients whom DJ catheter was performed during the period of pregnancy should be kept under the close follow-up and the patients who had the determination of bacterium in their urine culture should be treated with the appropriate antibiotics.

Key words: Double-J catheter, hydronephrosis, pregnancy, urinary tract infections.

Introduction

Hydroureteronephrosis during the period of pregnancy has often seen due to the hormonal and mechanical reasons. Increased estrogen during pregnancy, progestational hormones and prostaglandin-like agents have been able to lead to the dilation in the urinary tract without obstruction (1). The dilatation starting at 6th to 10th weeks of pregnancy may become visible in ultrasonography by the 26th to 28th weeks at 90% of the pregnant women (2). It has been reported that the urinary tract dilation could be seen at about 50% in 2nd and 3rd trimester of pregnancy. It has been reported that the dilatation has not had an increase until the 30th week of the gestation and then has remained constant (3). The conservative methods of the treatment are preferred unless it is compulsory in the hydronephrosis of pregnancy. More invasive treatments such as ureteral catheterization, percutaneous nephrostomy, ureteroscopy or ureteroscopic lithotripsy can be performed despite the conservative approach, if there is no decline in the symptoms.

The bacteria form the infections more easily in the urinary tract as a result of the difference of urinary content during pregnancy and some hormonal and physiological changes. The prevalence of asymptomatic bacteriuria in pregnant women varies between 4 and 7%. (4) The risk of the transformation of asymptomatic bacteriuria to the symptomatic

infection is 3-4 times higher compared with those who are not pregnant. (4.5) Pyelonephritis especially seems in the 3th trimester where the hydronephrosis and stasis in urinary tract are the most (6).

The method of the treatment which has widely been applied is the placement of ureteral Double-J (DJ) catheter in the treatment of symptomatic hydronephrosis which resistant to the conservative treatment seen in pregnants. Hydronephrosis and urine stasis are the most common causes of the urinary tract infections seen during the period of pregnancy. Since the placement of ureteral DJ catheter referred in the treatment of hydronephrosis of pregnancy prevents the urinary stasis and hydroureteronephrosis, ureteral DJ catheter may lead to the development of urinary tract infection since it is a foreign body though it reduces the development of urinary tract infection.

Materials and methods

Admitted to our clinic because of the hydronephrosis of symptomatic pregnancy and performed the ureteral DJ catheter, 42 patients were included to our study. Symptomatic hydronephrosis of pregnancy was defined as a stone disease presenting with urinary obstruction, renal colic and pyelonephritis. Assessed with ultrasound, the degree of hydronephrosis in all of the patients was determined with a schedule detailed by Zwergel and et al. Based on the maximal calyceal diameter, the degree of hydronephrosis was determined as 5-10, 10-15 and> 15 mm of mild, moderate and marked hydronephrosis, respectively (6). The patients were evaluated with the complete urinalysis, urine culture, serum urea and creatinine as well as ultrasonography before and after the application of the ureteral DJ catheter. The patients who had the urinary tract infection were excluded from the study before the ureteral DJ catheter was hooked up. Conservative treatment consisted of hydration and analgesics, when they are needed, were performed to all of the patients. The patients whom the ureteral DJ catheter was performed due to the hydronephrosis of pregnancy were evaluated with their symptoms and urine cultures in terms of urinary tract infection after two weeks then the application. Urinary tract infections were classified as asymptomatic bacteriuria, acute cystitis and acute pyelonephritis according to the breeding in the symptom and urine culture.

Results

42 patients whit hydronephrosis and who the ureteral DJ catheter was administered were included to the study. The average weeks of their pregnancy were determined as 27.2 ± 6.8. Two patients were in the first trimester (4.8%), 14 patients were in the second trimester (33.3%) and 26 patients were in the third trimester (61.9%). The stone disease was detected in fourteen patients (33.3%). The ages of the patients were between 18 and 37 (mean 26.8). The average number of pregnancies in pregnant women who were taken to the study was found as 1.76. It was the first pregnancy of twenty-four patients (57.1%). Hydronephrosis of grade II was detected in 24 renal units and hydronephrosis of grade III was detected in 18 renal units. There was right hydronephrosis in 28 patients, left hydronephrosis in 13 patients, bilateral hydronephrosis in one patient. The characteristics belonging to the patients are shown in Table 1. Since the right side was symptomatic, DJ catheter was inserted just to the right of the patient with bilateral hydronephrosis. There was no the determination of bacterium in urine culture of 30 (71.4%) of 42 patients who the ureteral DJ catheter was performed. There was the determination of bacterium in urine culture of 12 patients (28.6%). It was detected that there were asymptomatic bacteriuria in 5 patients (12%), acute cystitis in 4 patients (9.5%), and acute pyelonephritis in 3 patients (7.1%). The patients who developed infection were started the appropriate treatment of antibiotics according to the result of culture-antibiogram and they were kept under close follow-up, being proposed plenty of hydration. Eschenichia coli (E. coli) was reproduced at 10 of 12 patients who had the determination of bacterium in urine culture, Klebsiella pneumoniae (K. pneumoniae) in one patient and Pseudomonas aeroginosa (P.aeroginosa) in one patient were reproduced. The distribution of the patients whom the ureteral DJ catheter was performed has been given in Table 1 in terms of the urinary tract infections.

Table 1. Characteristics of the patients taken to the study and the distribution of urinary tract infections which are seen

	Complete group	The determination of bacterium in urine culture (+)			The determination of bacterium in urine culture (-)
		Asymptomatic bacteriuria	Acute cystitis	Acute pyelonephritis	
Number of the case	42	5	4	3	30
Age (Year)	26.8±4.9	24.9±3.8			27.4±4.2
Number of pregnancy	1.76±0.87	1.82±0.79			1.71±0.81
Week of pregnancy	27.2±6.8	26.9±6.4			27.5±6.1
Side of hydronephrosis	Right:28 Left:13 Bilateral:1	Right:8 Left:4			Right:20 Left:9 Bil:1
Degree of hydronephrosis	GradeII:24 GradeIII:18	GradeII:7 GradeIII:5			GradeII:17 GradeIII:13
Serum creatinine(m/dl)	0.69±0.42	0.71±0.38			0.68±0.40
Active pathogen	E.coli:10, P.Aeroginosa:1, K.Pneumonia:1				-

Discussion

Hydronephrosis is a common situation in pregnancy. Hydronephrosis of pregnancy usually improves within 48 hours after the birth. The improvement has lasted for 2-12 weeks in some pregnants. (7,8) Hydronephrosis of pregnancy has seemed in the right kidney three times more common in the left since the right ureter intercrossed the iliac vessels more proximally and the left ureter of the sigmoid colon in the left side relatively protected from the pressure. They are accepted as normal and the intervention is not usually required as hydronephrosis of grade I- II seen in the right kidney were not symptomatic (3). The right hydronephrosis was also available at 66.7% of our cases.

It is important to protect the fetus from radiation during pregnancy. It has been reported that one rad X-ray intake has 2 to 4 times increased the development of the risk of malignancy in the age of childhood (2). The most risky period is the first trimester in terms of radiation. For this reason, the USG should be preferred as the first diagnostic method in the assessment of pregnancy of hydronephrosis. Ultrasonography (USG) should be the first method to be applied in order to check whether the DJ catheter is in its place in pregnants with hydronephrosis whom the ureteral DJ catheter was performed. In a study belonging to Hellawell and et al, it has been reported that there is not any drawback in order to control whether a single dose of X-ray and the DJ catheter have been in their place (9). When the ultrasound is insufficient, the magnetic resonance imaging (MRI) should be the method to be refered since it does not require the contrast substance and does not contain the radiation. We used the ultrasound in all of our cases in order to watch whether the DJ catheter was in its place and to monitor the degree of hydronephrosis.

Today, the double-J catheters have a widespread field of the application in urology. Stone-related obstructive events, iatrogenic ureteral and pelvic injuries, urological plastic surgeries, large kidney stones before extracorporeal shock wave lithotripsy (ESWL), ureteral obstructions due to the reasons of benign and malignant, ureteral fistula and pregnancy of hydronephrosis can be considered as the main areas of the application (10,11,12,13). DJ catheters are usually placed in the ureter in endoscopic and they provide the continuity of urine passage between the kidney and bladder. Thus, blocking the urinary stasis, they help decline the existing hydronephrosis and help improve existing urinary infection.

The procedures of DJ catheter placement were carried out under local anesthesia (lidocaine gel). Prophylactic antibiotics (penicillin derivatives) was given to every patient whom the interventional therapy was performed. All of the patients were followed with the complete urinalysis, urine

culture and abdominal ultrasound. Antibiotic therapy was started to the patients with symptomatic and who had the determination of bacterium in their urine cultures according to the result of antibiogram. DJ catheters were replaced with a new one when needed during pregnancy. 15 days after the birth, the patients were taken for the evaluation and their upper urinary systems were controlled with the intravenous pyeleography by imaging DJ catheters of the stone-free the patients. The patients with stones were treated with an appropriate method of ESWL, ureterorenoscopy (URS) or percutaneous nephrolithhotomy (PCNL) according to the size and localization of the stone.

Hydronephrosis of pregnancy in our study has been seen more often in nulliparous as in other studies (14). The average number of pregnancy is as a high ratio as 1.76.

Pregnancy is not a predisposing factor for the formation of the stone. The incidence of stone disease in pregnant women is not more than non-pregnant women. However, the hydronephrosis accompanied by the clinical conditions such as renal colic, hematuria, urinary tract infection is more frequently emerged since the physiological dilatation of the ureters during pregnancy and the changes of backflow due to the pressure of the uterus provide more opportunity of the mobility to the stone.

The bacteria form the infections more easily in the urinary tract as a result of some hormonal and physiological changes and the difference of the urinary content during pregnancy. 4-6 % of the women in childbearing age is bacteriuric. (14) The prevalence of asymptomatic bacteriuria in pregnant women varies between 4-7%. (4) In fact, the incidence is not different from the non-pregnant as it is seen from these figures. (6,15) However, the replay attack is more common in these pregnants (4). The risk of the transformation of asymptomatic bacteriuria to the symptomatic infection is 3-4 times higher compared with those who are not pregnant. (4,5) The risk of bacteriuria increases in parallel during pregnancy (6). Acute pyelonephritis develops in the later stages of pregnancy in as many as 20-40% of untreated bacteriuria in early pregnancy. On the contrary, acute infection develops in less than 1% of the people who do not have infection in early pregnancy. Thus, most of the acute pyelonephritis cases can be prevented with the detection of asymptomatic bacteriuria in early stages of pregnancy. (4) Pyelonephritis seems in the 3rd trimester where especially the hydronephrosis and the stasis in the urethra are maximum (6). The cases that the response is inadequate to the therapy are generally the upper urinary tract infections. (4) 25-30 % of asymptomatic bacteriuria in pregnancy progressively forms the symptomatic urinary tract infection. In addition, urinary tract infections during pregnancy have adverse effects on the fetus. The rate of premature birth and low-birth newborns are higher in bacteriuric pregnants. (4,5) The prevalence of bacteriuria seen in pregnancy increases as related to many factors such as the duration of pregnancy, multiparity, low socioeconomic status, age, sexual activity, diabetes mellitus, sickle celled-anemia and the history of urinary tract infection in the past. (4, 5) Researchers have reported that the elimination of bacteriuria reduced the incidence of prematurity (16,17). Monitored during pregnancy, the most common pathogen that we encountered in asymptomatic bacteriuria, acute cystitis and acute pyelonephritis is E. coli.

There was the determination of bacterium in urine culture of 12 of 42 cases who we evaluated. While 3 patients with pyelonephritis were hospitalized, the other patients were started the appropriate antibiotic therapy as outpatients and they were kept under close follow-up with the complete urinalysis and urine culture. All of the patients who had the determination of bacterium in urine culture responded to the antibiotherapy which was given.

Application of ureteral DJ catheter is a frequently used method in the treatment of hydronephrosis of symptomatic pregnancy which did not respond to the conservative treatment. In a study in our country, it has been reported that 53.8% of 26 patients with hydronephrosis of symptomatic pregnancy did not response to the conservative therapy and ureteral DJ catheter was performed to them. (18) Application of ureteral DJ catheter in the treatment of hydronephrosis of symptomatic pregnancy which did not respond to the conservative treatment is effective in the improving of renal colic and urinary tract infections. Urinary infections such as acute pyelonephritis accompanied by hydronephrosis of pregnancy can lead to the abortion or premature action according to the stage of pregnancy. Although the application of ure-

teral DJ catheter is effective in the prevention of these complications which will occur, urinary tract infections can also be seen after the application of ureteral DJ catheter.

Conclusions

In the treatment of hydronephrosis of symptomatic pregnancy which did not respond to the conservative treatment, urinary tract infections can be seen in different groups from asymptomatic bacteriuria to the acute pyelonephritis which can lead to the complications such as pregnancy-related abortion and premature action after the application of ureteral DJ catheter. Therefore, the patients, whom ureteral DJ catheter was performed during the period of pregnancy, should be kept under close follow-up and the patients who had the determination of bacterium in their urine culture should be treated with the appropriate antibiotics.

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Effect of heart rate control using metoprolol on serum hypersensitive C-reactive protein in patients with chronic persistent atrial fibrillation

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Abstract

Objective: Oral metoprolol was used to control the heart rate (HR) of patients with chronic persistent atrial fibrillation (AF) and serum hyper-sensitive C-reactive protein (hs-CRP) was measured aiming to investigate the effect of HR control on inflammation.

Methods: On the basis of resting HR, 130 patients with chronic persistent AF were divided into high HR group (≥90 beats/min) and low HR group (< 90 beats/min). In the control group, 20 subjects with sinus rhythm were recruited. In the high HR group, patients were given oral metoprolol (50 mg/d) besides routine therapy. Before treatment and one month after treatment, fasting venous blood was collected, and turbindimetric immunoassay was performed to measure the content of serum hs-CRP.

Results: All subjects completed follow-up. In high HR group, metoprolol treatment was not discontinued due to resting HR of <60 beats/min. At 1 month after treatment, the HR in the high HR group was markedly reduced (110±19 vs 83±25 beats/min, P<0.01), and the clinical symptoms were markedly improved. Before treatment, the hs-CRP in high HR group was significantly higher than that in low HR group (9.23±4.39 VS 5.97±2.85 mg/l, P<0.01), but there was no marked difference in hs-CRP between two groups after treatment. In the high HR group, the hs-CRP in patients with HR of ≥90 beats/min was dramatically higher than that in those with HR of <90 beats/min (P<0.01).

Conclusion: In chronic persistent AF patients, the serum hs-CRP is related to resting HR, and the hs-CRP in patients with HR of ≥90 beats/min is higher than that in those with HR of <90 beats/min. In patients with HR of ≥90 beats/min, the hs-CRP is markedly reduced following HR control with metoprolol, accompanied by improvement of clinical symptoms. These findings suggest HR control can attenuate inflammation in patients with chronic persistent AF.

Key words: Atrial fibrillation, heart rate, C-reactive protein.

Introduction

Atrial fibrillation (AF) is one of the most common tachyarrhythmias. In recent years, researchers find that inflammation plays an important role in the occurrence and development of AF[1,2]. C-reactive protein (CRP) is an important marker for inflammation. Numerous studies have confirmed that AF can induce the elevation of C-reactive protein [3]. To date, some studies have shown that the improvement of AF is accompanied by attenuation of inflammation, but the effect of heart rate (HR) control on CRP is less evaluated in patients with AF. In the present study, metoprolol was used to control the HR of patients with chronic persistent AF and the serum hyper-sensitive CRP (hs-CRP) was measured aiming to investigate the effect of HR control on inflammation.

Subjects and methods

Subjects

A total of 130 patients with chronic persistent AF were recruited from the Department of Cardiology of our hospital from March 2011 to March 2012, and these patients were diagnosed with primary AF or AF secondary to hypertension. The chronic persistent AF was defined according to the criteria in the Guideline for Treatment of Atrial

Fibrillation developed by the European Cardiology Society (ECS) in 2010 [4]. On the basis of resting HR, patients were divided into high HR group (HR ≥90 beats/min) and low HR group (HR <90 beats/min). AF was diagnosed by 12-lead ECG. Exclusion criteria: 1) Patients had a history of heart failure, echocardiography showed the left ventricular ejection fraction of <50%; 2) Patients with valvular heart disease, hypertrophic cardiomyopathy, hyperthyroidism induced heart disease or with myocardial infarction within 3 months were excluded; 3) Patients with severe conduction block due to lesions in the atrioventricular node and sinoatrial node were excluded; 4) Patients recently developed infection, tumor, hematological diseases, autoimmune diseases, severe liver and/or kidney dysfunction, or were treated with anti-inflammatory drugs / anti-oxidants, or with statins / complex vitamins; 5) Patients having low blood pressure (< 90/60 mmHg), electrolyte imbalance, digitalis poisoning, bronchial spasm or evident lesions in other organs were excluded. All patients received physical examination, chest X ray, 12-lead ECG, 24-h ambulatory electrocardiography, cardiac ultrasonography, and detection of liver and kidney function, electrolytes and thyroid hormone. In addition, 20 volunteers without AF history matched with subjects in the high HR group were recruited as controls.

Control of heart rate

Besides routine treatment, patients in the high HR group were given oral metoprolol (AstraZeneca) at 6.25 mg twice daily. When side effects were absent, oral metoprolol was administered at 12.5 mg twice daily since day 3. After treatment for 6 days, when the resting HR was ≥60 beats/min and blood pressure was ≥100/60 mmHg, metoprolol was administered at 25 mg, twice daily for maintenance therapy. Before and after treatment, the blood pressure, HR, clinical symptoms and signs were recorded. On the basis of criteria developed by the European Heart Rhythm Association (EHRA) [4], the clinical symptoms of these patients were graded. The ECG was analyzed and adverse events were recorded. Once adverse effect was present or the ventricular rate was <60 beats/min or hypotension was present, study was stopped. In the low HR group, HR control was not performed. Routine therapy was done with aspirin enteric-coated tablet (100 mg/d), and calcium antagonist or ACEI/ARB (for hypertension patients).

Detection of hs-CRP

One day before treatment and 1 month after treatment, fasting venous blood was collected (3 ml) and centrifuged at 2500 r/min for 10 min. The serum was collected and turbindimetric immunoassay was performed to measure the hs-CRP content.

Statistical analysis

All quantitative data were expressed as means ± standard deviation ($\bar{x} \pm s$). Comparisons between 2 groups were done with independent t test and those of data before and after treatment with paired t test. Qualitative data were compared with chi square test. A value of $P<0.05$ was considered statistically significant. Statistical analysis was done with SAS version 6.12.

Results

Demographics

All patients completed the follow up. In the high HR group, the dose of metoprolol reached 50 mg per day. Treatment was not discontinued in the high HR group due to ventricular rate of <60 beats/min or blood pressure of <90/60 mmHg. The demographics of these subjects are shown in Table 1.

HR and clinical symptoms

On the basis of criteria developed by EHRA, the clinical symptoms were graded. In the high HR group, the HR was markedly reduced after treatment accompanied by evidence improvement of clinical symptoms ($P<0.01$). However, in the low HR group, the HR and clinical symptoms remained unchanged. After treatment, there were no significant differences in the HR and the grade of clinical symptoms between high HR group and low H group. The HR and grade of clinical symptoms are shown in Table 2.

hs-CRP

The hs-CRP level in patients with AF was markedly higher than that in controls ($P<0.01$), and the hs-CRP level in high HR group was significantly increased when compared with the low HR group ($P<0.01$). After treatment, the hs-CRP level in the

Table 1. Demographics of subjects in three groups ($\bar{x}$ ±s)

	n	Mean age (yr)	Gender (M/F)	Mean HR (beats/min)	Left atrial diameter (mm)	LVEF (%)	Duration of AF	Hypertension patients (n)
Control	20	59.2±9.7	12/6	75±15	36.3±4.9	62.2±6.5	-	14 (70%)
Low HR	60	61.3±8.9	60/30	78±12	41.3±5.0*	60.6±7.1	2.8±1.6	39 (65%)
High HR	70	60.6±9.2	70/38	110±19D	42.0±4.9*	59.4±5.9	2.5±1.5	49 (70%)

*Note: * P<0.01 vs control group; Δ P<0.01 vs low HR group*

Table 2. HR and grade of clinical symptoms in two groups ($\bar{x}$ ±s)

Group	n	HR before treatment (beats/min)	HR after treatment (beats/min)	Grade of symptoms before treatment I II III IV	Grade of symptoms after treatment I II III IV
Low HR	60	78±12	75±16	28 25 7 0	29 26 5 0
High HR	70	110±19*	83±25 D	19 33 18 0	32 28 10 0 D

*Note: * P<0.01 vs control group; Δ P<0.01 vs before treatment*

high HR group was comparable to that in the low HR group. The hs-CRP level in the low HR group remained unchanged after treatment. In the high HR group, the hs-CRP level in patients with HR of ≥90 beats/min was dramatically higher than that in those with HR of <90 beats/min (P<0.01). The hs-CRP level before and after treatment is shown in Table 3 and 4.

Table 3. hs-CRP level in different groups ($\bar{x}$ ±s) (mg/l)

Group	n	Before treatment	After treatment
Control	20	2.30±1.13	-
Low HR	54	5.97±2.85*	5.92±2.91
High HR	66	9.23±4.39*Δ	6.49±3.17**

*Note: * P<0.01 vs control group; ** P<0.01 vs before treatment; Δ P<0.01 vs low HR group*

Table 4. hs-CRP level of patients with AF before and after treatment ($\bar{x}$ ±s) (mg/l)

Group	HR	n	Before treatment	After treatment
Low HR	80-89	23	6.21±3.03	6.10±2.98
	70-79	19	5.85±2.64	5.81±2.69
	60-69	18	5.79±2.70	5.82±2.91
High HR	≥90	16	9.23±4.59	8.34±3.43
	80-89	19		6.01±3.01*
	70-79	20		5.89±2.74*
	60-69	15		5.91±2.80*

*Note: * P<0.01 vs patients with HR of ≥90 beats/min in high HR group*

Discussion

AF is a common arrhythmia and its pathogenesis is still unclear. Besides the multiple reentrant wavelet hypotheses, inflammation is closely related to the occurrence of AF and may serve as a pathogenic factor of AF[5]. CRP is an important marker of inflammation and might involve in the occurrence and development of AF[6]. In the present study, metoprolol was used to control the HR of patients with chronic persistent AF, and the serum hs-CRP was measured to explore the effect of HR control on inflammation in these patients. Turbindimetric immunoassay has higher accuracy when the serum hs-CRP is at a low level (0.15-10mg/L). Our results showed: 1) The hs-CRP level in patients with chronic persistent AF was significantly higher than that in controls; the hs-CRP level in AF patients with high HR (≥90 beats/min) was markedly increased when compared with those with low HR (<90 beats/min); 2) After HR control by metoprolol (50mg/d), the hs-CRP level in patients with high HR was markedly reduced accompanied by improvement of clinical symptoms, but the hs-CRP level in patients with HR of ≥90 beats/min after treatment was significantly higher than that in those with HR of <90 beats/min.

CRP is an indicator of inflammation and a typical acute phase protein synthesized in the liver [7]. CRP can activate the CRP receptor on the monocytes and granulocytes directly (infiltration or aggregation) or indirectly resulting in production of cytokines including IL-6 and tumor necrosis fac-

tor. These cytokines may induce the fracture of plaques. Studies have demonstrated that, at 6~12 h after inflammation or injury, the blood CRP is significantly increased and CRP can serve as an indicator of focal inflammation with high sensitivity and accuracy. The activated inflammatory cells and vascular cells can secret excessive pro-inflammatory cytokine IL-6 which then stimulates the hepatocytes to produce a large amount of CRP leading to a cascade reaction. This may induce or aggravate inflammation. Available studies have shown the correlation between CRP and the occurrence and maintenance of AF and recurrence of AF following cardioversion, and CRP can be used to predict the occurrence and persistence of AF [8-10]. The roles of CRP in AF are as follows: 1) the blood CRP directly involves in the focal inflammation. CRP can bind to the receptor on myocytes activating the complement pathway, which may directly cause damage to the myocytes. 2) Apoptosis plays an important role in the pathogenesis of AF. Increase in apoptosis and interstitial fibrosis may lead to atrial structural remodeling. In the regulation of apoptosis, CRP may function as opsonin. CRP involves in the clearance of apoptotic cells, promotes the reduction of total atrial cells and aggravates interstitial fibrosis [11]. Recently, Chang et al [10] found that CRP could significantly increase the inward L-type calcium current in atrial cells, which was found to be closely related to the occurrence of AF. Our results indicated that the hs-CRP level in patients with chronic persistent AF was markedly higher than that in controls with sinus rhythm, which was consistent with previously reported [1]. In addition, the relationship between HR and CRP was also evaluated. Results showed the hs-CRP in patients with high HR (≥90 beats/min) was significantly higher than that in those with low HR (<90 beats/min). Furthermore, study reported that the CRP level was reduced and the frequency of AF also decreased after treated with glucocorticoids for anti-inflammation in patients with paroxysmal AF [12]. This suggests that reduction of serum CRP might be beneficial for the reduction of AF frequency or attenuation of AF persistence. Some studies have confirmed that the CRP can reduce after cardioversion [2]. In the present study, HR was controlled and then results indicated that the hs-CRP in chronic AF patients with HR of ≥90 beats/min was markedly lower than that in those with low HR (<90 beats/min). This suggests that control of ventricular rate may reduce CRP level and then attenuate the inflammation.

In 2010, the Guideline for Treatment of AF (ESC) proposed on the basis of findings in the study of Van Gelder et al[13] that loose HR control (resting HR<110 beats/min) is as effective as strict HR control (resting HR<80 beats/min; HR under moderate excise <110 beats/min) and has similar influence on prognosis. However, relative high HR is a key factor causing clinical symptoms. In the study of Van Gelder et al, the resting HR was loosely controlled at <110 beats/min. Although the compliance rate is at a high level (97.7%), this goal is too loose. In the present study, patients with HR of ≥90 beats/min were given metoprolol at 50 mg/d to control the HR, and the compliance rate (resting HR < 90 beats/min) was 77.1% (54/70). On the basis of classification of EHRA, the clinicam symptoms were markedly improved. After treatment, there was no pronounced difference in the HR and the grade of clinical symptoms between patients in the high HR group and those in the low HR group. HR is one of indicators following HR control, and improvement of clinical symptoms and attenuation of inflammation should also be taken into accounted. Our results suggests, among patients with high HR (resting HR≥90 beats/min), increase in markers of inflammation implies the requirement of HR control, and metoprolol may be an alternative drug.

In the present study, we only investigated the influence of HR control on inflammation. Currently, statins are widely applied in the treatment of cardiovascular diseases and have been demonstrated to be beneficial for the suppression of inflammation. In addition, we did not evaluate the influence of blood pressure control on hs-CRP, which may bias our findings. Moreover, the sample size in this study was small and the time points used for detection were randomly selected. Thus, in future studies, the time points for detections should be carefully designed and the sample size be increased aiming to get a conclusion on the influence of HR control on inflammation in AF patients.

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Functional endoscopic sinus surgery course based on motor skills training

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Abstract

Objective: To evaluate the effects of functional endoscopic sinus surgery training performed on human cadaver and measure the perceptions of the course participants

Study design: Descriptive study.

Methods: Study includes a total of 30 participants who are specialists, associates or research assistants in Akdeniz University Faculty of Medicine, Antalya. A preliminary 15 item test was applied to participants. Course consists of three stages. In the first step, theoretical content was provided. Second step was the demonstration of the lecturer on human cadaver. At the last step, participants perform the practice on cadaver on their own. At the end of the course, a final test was applied and course evaluation forms were filled by the participants.

Conclusion: We can conclude that FESS training performed on cadaver provides a safe and effective learning environment for physicians before they meet with real patients in clinical settings.

Key words: Motor skills, endoscopic sinus surgery, cadaver.

Introduction

The use of simulators in undergraduate, postgraduate and continuing medical education evidently increased since 1990s in order to ensure patient safety and reduce medical errors. Emphasize on patient safety in surgical education underlines the fact that simulation based training is essential until the surgeons become proficient.[1,2]

Simulation is defined as the imitating of things and concepts such as tasks, relationships, phenomenon, equipment, behavior or cognitive activities that are present in reality.[3] Simulators play important roles in surgical education not only because they enable repetitive practice for developing necessary surgical skills and diminishing the stress level of surgeons but also reducing the anxiety about harming patients' well-being.[4]

Since Messerklinger highlighted the importance of ostiomeatal complex, functional endoscopic sinus surgery (FESS) technique increasingly performed for chronic sinusitis, recurrent sinusitis and nasal polyposis.[5,6] Widespread use of FESS caused an associated increase in complication rates. Due to the relative difficulty of FESS technique, the complication rates vary between 5-17%.[7-9] The complications those are most frequently associated with FESS are: anterior ethmoid artery trauma, lamina papyracea trauma, nasolacrimal duct and/or sac trauma, sphenopalatine artery trauma, optic nerve trauma, skull base trauma leading to cerebrospinal fluid (CSF) leakage, posterior ethmoid artery trauma and medial rectus muscle injury.[10-12] In order to minimize the complication risk, it is recommended to practice with endoscopic sinus surgery simulators, human cadaver or animal models before starting real surgical practices.[13-14]

The course given in our medical faculty focuses on teaching the anatomical links of the paranasal sinuses and strategies about preventing FESS related complications. Theoretic and practical cadaver practices on surgical techniques those are used during dissection are also performed. The purpose of this study is to evaluate the FESS training performed on cadaver and measure the perceptions of the course participants.

Materials and methods

This descriptive study includes all specialists, associates and research assistants who participated in applied cadaver endoscopic surgery course which was held during 2011 (n=30).

The course started with the application of a 15 item preliminary test. Questions were related to anatomy, otorhinolaryngology, radiology and neu-

rosurgery. Participants answered the questions but were not asked for their names. Following the end of the course, a form was filled by each participant as the final evaluation instrument.

Course program consists of three stages. At the first stage, there are theoretical interactive lessons about the anatomy of endoscopic sinus surgery, otorhinolaryngology, radiology and neurosurgery. At the second stage, participants monitored the lecturer while the lecturer performed FESS on the cadaver. Lecturer completed the procedure describing every step and the participants had the chance to observe and ask questions. At the third and the last stage, basic motor skills are being developed. Participants perform every step of the procedure on the cadaver in clinical anatomy laboratory settings. During this stage, lecturer assists the participants and evaluates their performances.

At the end of the course, a final test was applied and course evaluation forms were filled by the participants. Evaluation form consists of 10 questions. Participants were asked if they have attended any similar courses before. They were also asked to evaluate the facilities, used models, cadaver dissection and contents of the presentations based on a five-point Likert scale (1= very bad, 5= very good). Ten-point Likert scale was used to measure if the course and cadaver dissection practices met paticipants' expectations (1=did not meet the expectations at all, 10=perfectly met the expectations). They were also asked if they can perform the procedure on their own with a ten-point Likert scale. Last questions were about if they would recommend the course to other colleagues and believe that the course should be a permanent component of otorhinolaryngology education.

All statistical analyses were done with SPSS software (Version 13.0). The sum of correct answers both at the preliminary and final tests were calculated and their means were compared to evaluate the course program. Paired samples t test was used to evaluate the level of correctly answered questions with respect to independent variables. Reclassification was done for ten-point Likert scale items: 1 to 4= not satisfactory; 5 to 7= undecided; 8 to 10= very good.

Results

Of the total 30 participants, 13.8% were associates, 70.2% were specialists and 13.3% were research assistants. 43.3% of the participants reported that they have attended similar courses before while for 56.7% of them this was their first course. Figure 1 shows whether the expectations of the participants about the content of the course were met. 73.3% of the participants reported that their expectations were fully satisfied.

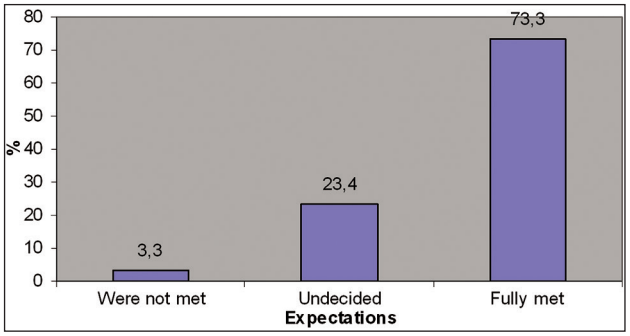

Figure 1. The distribution of participants based on their opinions on whether the course met their expectations

In Table 1, the distribution of the answers of participants to questions about course facilities, used models, cadaver dissection and presentations are given. Almost all participants are satisfied with the course facilities and contents of presentations.

Two participants (6.7%) reported that they will not be able to perform the procedure on their own with real patients (Figure 2).

Table 1. Evaluation of the course with respect to several components

	Bad – Very Bad		Good – Very Good	
	n	%	n	%
Facilities	1	3.3	29	96.7
Used models	2	6.7	28	93.3
Cadaver dissection	1	3.3	29	96.7
Content of the presentations	0	0	30	100.0
Visual and audial tools used in presentations	0	0	30	100.0

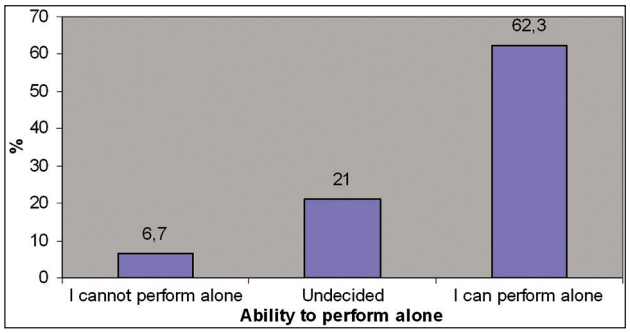

Figure 2. The distribution of participants who believe that they can perform the procedure on their own after attending endoscopic sinus surgery training

The percentage of participants who were fully satisfied with cadaver dissection practices was 68% (Figure3).

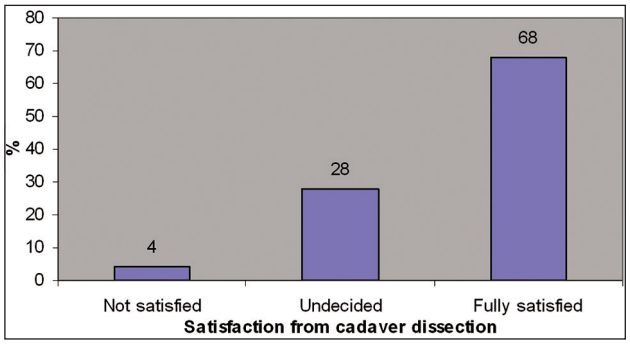

Figure 3. Satisfaction from cadaver dissection

A total of 83.3% of the participants stated that they would recommend the course to their colleagues while 96.6% of them reported that the course should be a permanent component of otorhinolaryngology education.

Table 2 shows the preliminary and final test scores of the participants. The mean score was 6.5 ± 2.6 before the course and it significantly increased to 12.6 ± 0.4 after the course (Table 2).

Discussion

FESS is the standard surgical procedure that is used in nasal and paranasal pathologies. Despite the advances in technology, complication rates vary considerably.[8, 9] Previous studies reported that the main determinant of complication rates is the experience level of the surgeon. Complication rates tend to decrease if the surgeons are more experienced. In order to achieve proficiency in basic motor skills, repetition of the practice is also as crucial as fundamental knowledge.[8] Although there are several methods in surgical training to acquire proficiency and excellence for technical skills, medical community accepts the use of simulators is the best way to ensure patient security.[15] FESS practice is a basic motor skills training. Zukkerman et.al. investigated the effect of cadaver dissection on FESS and showed that practicing with cadaver increases the success of the surgery and surgeon while decreases the complication rates.[13] The level of satisfaction of the physicians is also very high in our study. This can be related to the fact that human cadaver offers every details of human anatomy and tissue. Most important component of training with cadaver is to comply with the concept of "fidelity". This can be considered as strength of the cadaver practice: it reflects the reality. When the responses are reevaluated omitting the data collected from research assistants, all participants told that they can perform the procedure on real patients.

Related research show that structured surgical basic motor skills training increases the overall performance in surgeries.[16, 17] When the course program was formed, based on an integrated approach, we not only focused on the purpose of improving surgeons' skills but also aimed to compensate the lack of professional knowledge and provide the opportunity to practice on models and perform cadaver dissection. Almost all participants reported that they were fully satisfied with the course content and facilities. It is possible

Table 2. Preliminary and final test scores of the participants

Before the Course	After the Course	p
Mean ± S.D. (Min-Max)	Mean ± S.D. (Min-Max)	0.0001
6.5 ± 2.6 (1-12)	12.6 ± 0.4 (12-13)	

to conclude that the FESS course in our faculty achieved its goals in terms of addressing the fundamental needs of the surgeons.

When the performances of the participants prior and after the course were compared, there was a statistically significant increase. Participants reported that they were completely satisfied with the theoretical content of the program. Since the level of knowledge in a specific field covers the necessary skills and attitudes related with the field, it is important for the course to be able to achieve the goal of improving overall knowledge level of participants.

More than half of the participants reported that they would recommend the course to their colleagues since the program fully meets the pre-course expectations and the FESS procedure is beneficial for their profession. Except the research assistants, almost all physicians declared that they will be able to perform the procedure on real patients at their own clinical settings. This finding indicates that more than half of the participants achieved expertise in FESS after being involved in FESS course. Our results support the previous research on how simulators facilitate learning in surgical education. [18]

Conclusion

Majority of the course participants were satisfied with the content of the course. Lack of theoretical and practical skills which was determined with a preliminary test before the start of the course was eliminated. Approximately half of the participants believe that they can perform the procedure on their own with real patients. We can conclude that FESS training performed on cadaver provides a safe and effective learning environment for physicians before they meet with real patients in clinical settings.

Acknowledge

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Depression and co-morbid chronic illnesses in family practice

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Abstract

Background: The data showed that comorbidity between chronic physical conditions and depression is common and that people with chronic diseases are significantly more likely to suffer from depression than those without. Our objectives are to study the prevalence of depression in patients attending family medicine clinics and its relation with patient's characteristics and chronic diseases.

Methods: This is a cross- sectional study based in family medicine clinics at the University of Jordan Hospital in Amman, Jordan. The study was done on 407 patients who voluntarily answered a self-administered questionnaire (October 2011 – January 2012). Our statistical analysis used Logistic regressions, odds ratio (OR), 95% confidence intervals (CI), and significance level of p- value <0.05.

Results: The Mean age was 40.38 with a standard deviation of 14.36. About half of the sample (49.8%) was less than forty years of age. Females constituted 66.1% of the sample. The prevalence of mild level of depression was 45.9%. Depressive symptoms were more likely to be found in those under the age of 40 (OR=2.5, 95% CI: 1.19-5.55), and those with a lower level of education designated as a high school diploma or less (OR=2.124, 95% CI: 1.043-4.326). Only patients with chronic headache and those with musculoskeletal disorders had a significant relationship with depression (OR=3.124, 95% CI: 1.111-8.782) and (OR=1.896, 95% CI: 1.078-3.332) respectively.

Conclusions: Depression is prevalent among our patients specifically who had chronic headache and musculoskeletal disorders. It was found that lower level of education level and younger than forty years of age are associated with higher prevalence of depression.

Key words: Depression, family practice, chronic diseases.

Introduction

Depression is an important international issue with public health implications due to its increased lifetime prevalence 2% to 15% and the risk of disability (1).

In effect, it is anticipated to become the second leading cause of morbidity by the year 2020 after heart disease (2).

Stemming from mental health promotion and development, treatment in the primary care setting(family practice) was recommended by the WHO report of 2001 (3). Depression is an important cause of morbidity as it follows a chronic and recurrent course with increasing disability if left untreated (4). Jordan is one of the developing country in the Middle East with a population of 6,249,000 (5). Majority of Jordanians populate the major cities and are highly urbanized.

Jordan is considered to be of middle -income country that would perceive an epidemiologic evolution, which is described by an increase of non-communicable diseases, especially heart and vascular disease, malignancy, diabetes, and chronic pulmonary conditions (6). In Jordan there are different health care systems including the ministry of health that is governmental, military system, university- based and the private sector. Primary health care (family practice) is mostly offered by the ministry of health and university- based sectors.

Evidence showed that chronic medical diseases are frequently linked with development of depressive symptoms and disorders.(7-10) and these comorbidities cause important impairment of health and their diagnosis becomes imperative in order to provide excellence care to patients (11, 12) . Several studies have also investigated the effect of intervention in depression on comorbid illnesses (13-16). few had proven that treating depression would improve certain outcomes in medical ill-

nesses such as pain and quality of life in arthritis patients(13) and glycemic control in diabetics(16).

Primary care physicians (family doctors) are in a position to evaluate patients' mental health and thus provide appropriate management systems (17) , particularly as they are principally providing care for chronically ill patients.

The objective of our study was to determine the prevalence of depression in patients attending family medicine clinics and to estimate the relationship of depression with a number of socio-demographic factors and chronic illnesses that are commonly seen in family medicine practice.

Methods

This is a cross sectional study which was conducted between October 2011 and January 2012 at the family medicine clinics at Jordan University Hospital (JUH) in Amman, Jordan.

Family medicine clinics are academic primary health care clinics that provide care for acute and chronic disorders and offer preventive care irrespective of patient's age or gender. JUH is a well-reputed establishment that serves patients, who are medically insured, and live in the capital Amman and the surrounding governorates.

The sample consisted of 407 patients, aged 20 years and over who attended the clinics for various reasons and completed a self-administered questionnaire. A research assistant was trained to ascertain that patients understood and answered all the questions appropriately. The exclusion criterion was any patient receiving antidepressant treatment.

The questionnaire used consisted of two parts. The first part was developed by the researchers and contained two sections. Section A included the participant's characteristics (age, sex, marital status and education level.) Section B questioned the participant if he/she had ever been diagnosed to have any of the chronic illnesses, which are mostly seen by family physicians. Chronic illness was defined as "health problems that require ongoing management over a period of years or decades" (18). Chronic diseases were grouped into chronic headache (migraine, chronic daily headache, tension headache), pulmonary diseases (asthma, chronic obstructed pulmonary disease, chronic sinusitis), coronary artery disease (CAD), in which [diabetic , hypertensive and dyslipidemic patients were included], thyroid diseases (hypothyroidism and hyperthyroidism), chronic skin disorders [eczema, psoriases] and musculoskeletal illnesses (including osteoarthritis, rheumatoid arthritis, fibromyalgia, chronic back pain). The presence of those chronic illnesses was confirmed by a review of medical records and medication intake. The number of diseases was counted by a simple count of chronic diseases in each patient.

The second part of the questionnaire consisted of the Arabic version of Beck Depression Inventory II (BDI II) scale, which consists of 21 groups of statements that are used for depression screening. This Arabic version has been validated and demonstrated to be culturally appropriate (19). BDI II is an update of the original BDI, which was altered to correspond to criteria from the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)(18). BDI II had shown excellent validity and reliability in multiple studies in different populations (20, 21) and in primary care patients (22).

Validity of the questionnaire was established in a pilot study on 50 patients, who were not included in the sample. The ethical scientific research committee at the faculty of medicine, University of Jordan, approved the study. Verbal informed consent was obtained from each participant.

The prevalence of depression was based on the result of Beck Depression Inventory II (BDI -II) scale, a total score of 13 or less was considered as normal, while a score of 14 and more was considered as depression.

Statistical analyses

The data was analyzed by using SPSS version 11.0 for Windows. Descriptive statistics were used for the main outcome of the study (sample characteristics and prevalence of depression).

Binary logistic regressions were used to estimate the sample characteristic that influences the prevalence of depression, using unadjusted odds ratio (OR) and 95% confidence intervals (CI). The statistical significance level of P -value less than 0.05 was used.

We calculated unadjusted and adjusted (models 1, 2), odds ratio (OR) and 95% confidence interval (CI) to study the association of chronic diseases and depression. In model 2 we adjusted for sex,

marital status, age, and education. Using unadjusted odds ratio (OR) and 95% confidence interval (CI), with P-value less than 0.05 to assess the relationship between the number of chronic disease and depression.

Results

Mean age of the studied sample was 40.38 with a standard deviation of 14.36.

Table 1 shows Almost half of the sample (49.8%) was less than forty years of age (20-39), while slightly more than one-third (37.6%) were aged 40-59 years. The remaining 12.5% were 60 years of age and more. Females constituted 66.1% of the sample. More than one third of the samples (32.2%) were single, divorced or widowed. Patients with high school education or less constituted 34.1%. Twenty percent of the sample achieved a two-year college education while 32 % were

Table 1. Sample Characteristics in numbers and percentage

		Number	Percent %
AGE	<=29	119	29.2
	30-39	84	20.6
	40-49	92	22.6
	50-59	61	15.0
	>=60	51	12.5
SEX	Male	138	33.9
	Female	269	66.1
Marital status	Married	276	67.8
	not Married	131	32.2
Educational level	High school or less	139	34.1
	2 years college	84	20.6
	Bachelor degree	132	32.4
	postgraduate	52	12.8
Depression	<14	220	54.1
(BDI II score)	>=14	187	45.9

Table 2. Association between the sample characteristics and prevalence of depression

Sample		Total	Depression			
			Yes(187)	No(220)	Unadjusted OR	P value
			N %	N %	OR† (95% CI)‡	
Age	<29	119	66 (55.5)	53 (44.5)	2.580 (1.193-5.580)	.016*
	30-39	84	43 (51.2)	41 (48.8)	2.568 (1.194-5.524)	.016*
	40-49	92	42 (45.8)	50 (54.3)	1.944 (.903-4.186)	.089
	50-59	61	21 (34.4)	40 (65.6)	1.242 (.539-2.860)	.611
	≥60	51	15 (29.4)	36 (70.6)	.	.
Sex	Male	138	53 (38.4)	85 (61.6)	.696 (0.446-1.086)	.110
	Female	269	134 (49.8)	135 (50.2)	.	.
Marital	Married	276	114 (41.3)	162 (58.7)	.630 (0.367-1.082)	.094
status	un Married	131	73 (55.7)	58 (44.3)	.	.
Education	High school or less	139	75 (54.0)	64 (46.0)	2.124 (1.043-4.326)	.038*
Level	2 years college	84	40 (47.6)	44 (52.4)	1.605 (0.743-3.468)	.229
	Bachelor degree	132	55 (41.7)	77 (58.3)	1.056 (0.512-2.177)	.882
	postgraduate	52	17 (32.7)	35 (67.3)	.	.

OR† = odds ratio
(95%CI)‡ = 95%confidence interval
**significant P value less than 0.05*

bachelor holders and 12.8% of the sample completed a postgraduate education. Prevalence of depression was 45.9% [with 187 patients scored 14 and more at the BDI II questionnaire].

Table 2 shows that Depressive symptoms were two and a half times more likely to be found in those under the age of 40. (Patient's aged 29 and less were two and a half times more likely to be depressed than who are more than 40 years of age, (OR = 2.580, 95%CI: 1.193-5.580 with P-value of 0,016). Patients between the age 30 and 39 years were two and a half times more likely to be depressed than older ones (OR = 2.568, 95% CI: 1.194-5.524 P-value of 0,016). Patients with high school education or less were more likely to be depressed than higher educated patients OR = 2.124, 95%CI: 1.043-4.326) P-value=0.038. There was no statistical significant relationship between depression and gender or the marital status.

Table 3 shows that depression was found to be three times more common in patients having chronic headaches (OR =3.124, 95%CI: 1.11-8.78) P- value= 0.031 while nearly twice as likely in patients complaining of musculoskeletal disorders (OR of 1.896, 95%CI 1.078-3.332) P-value=0.026. There were no statistical significant relationship between depression and the other chronic diseases (coronary artery disease, pulmonary, skin, or thyroid diseases).

Table 3. Association between chronic diseases and depression

		N (%)	Depression		Model 1		Model 2	
			Yes	No				
			N %	N %	OR† (95%CI)‡	P value	OR† (95%CI)‡	P value
CAD††	Yes	93 (22.9%)	36 (38.7%)	57 (61.3%)	.591 (0.358-.973)	0.039	1.016 (0.560-1.842)	0.960
	No	314 (77.1%)	151 (48.1%)	163(51.9%)				
chronic Headache	Yes	20 (4.9%)	14 (70.0%)	6 (30.0%)	2.768 (1.012-7.569)	0.047	3.124 (1.111-8.782)	* 0.031**
	No	387 (95.1%)	173 (44.7%)	214(55.3%)				
pulmonary diseases	Yes	29 (7.1%)	15 (51.7%)	14(48.3%)	1.321 (0.599-2.915)	0.490	1.576 (0.697-3.563)	0.274
	No	378 (92.9%)	172 (45.5%)	206(54.5%)				
allergic diseases	Yes	72 (17.7%)	37 (50.7%)	36 (49.3%)	1.093 (0.646-1.850)	0.83	1.008 (0.585-1.739)	0.976
	No	334 (82.3%)	150 (44.9%)	184(55.1%)				
thyroid diseases	Yes	21 (8.2%)	10 (47.6%)	11 (52.4%)	0.873 (0.345-2.205)	0.740	.696 (0.268-1.811)	0.458
	No	386 (94.3%)	177 (45.9%)	209(54.1%)				
Skin diseases	Yes	25 (6.1%)	8 (32.0%)	17 (68.0%)	.518 (0.216-1.241)	0.140	.555 (0.224-1.376)	0.204
	No	382 (93.9%)	179 (46.9%)	203(53.1%)				
Musculosketal Diseases	Yes	91 (22.4%)	47 (51.6%)	44(48.4%)	1.463 (0.898-2.398)	0.131	1.896 (1.078-3.332)	* 0.026*
	No	316 (77.6%)	140 (44.3%)	176(55.7%)				

OR† = odds ratio
(95%CI)‡ = 95%confidence interval
Model 1: unadjusted
Model 2: adjusted for socio-demographic variables (sex, marital status, age education)
CAD††: coronary artery diseases
**P- value <0.05*

Table 4. Association between number of chronic diseases and depression

		Total	Depression			
			no	yes		
			220	187	OR† (95%CI) ‡	P value
No of chronic disease	≥3	29	12(41.4%)	17(58.6%)	0.645 (0.291-1.429)	0.28
	1-2	202	116(57.4%)	86(42.6%)	1.232 (0.820-1.849)	0.32
	No disease	179	92(52.3%)	84(47.7%)	-	

OR†= odd ratio (95% CI)‡ =95% confidence interval

Table 4 we found that 58.6% of patients with three or more chronic medical conditions had depression. However, the relationship between the number of illnesses and depression was not statistically significant.

Discussion

In our study the prevalence of depression was found to be 45.9%, which is relatively high in comparison with other studies (10, 23-27). A possible explanation for our relatively high prevalence could be that we used the BDI- II which is solely a screening questionnaire. In addition, we included mild cases of depression by using 14 as a cutoff point in BDI-II.

There were significant differences in the rate of occurrence of depression studied in primary health care settings in diverse regions of the world that varied between 4.5% to 41.9% (10, 23). This could be attributed to different study methodologies used or in the form of type of the tool, the depression cutoff point, the population sex and age or the setting in which the study was done.

In our study depression was found mostly prevalent among the younger age group {20-39 years}. This is similar to what was found in a number of studies (27-29), while others found no significant difference among age groups (23, 24). The higher incidence of depression among the younger population could be related to the changes of the social and economic situation. Over the past few years the economic decline had burdened the general population. Our theory is that the younger age group did not possess the skills or ability to cope with these stressful times as compared to a better established more mature population. It is important to consider that this could be simply related to our sample and further studies are needed to validate our results.

With regards to education, patients who were illiterate or with a lower level of education (high school or less) were more likely to be depressed. A similar relationship was found in other studies (10, 30). Francis et al (31) reported that "there was a significant improvement in depression symptoms after one year of adult literacy program". It was hypothesized that "self efficacy" improved with education and this improved their depression scale scores, other researchers found higher prevalence of depression among patients with higher levels of education (27, 28).

Despite the fact that a number of other studies found the female gender to be a risk factor for depression (26-28), the relationship between female gender and depression was not statistically significant in our study.

With regards to marital status, the prevalence of depression varied in the literature from an increase in the married population(27, 32) to a more prevalent in single, widowed or divorced, (26, 28). We found that depression prevalence was 55.7% in patients who were single, widowed or unmarried but this was not a statistically significant finding.

Among the list of chronic illnesses studied, patients with headache and musculoskeletal disorders were found to be more likely depressed than patients without these illnesses. Population-based studies have revealed association between migraine, anxiety disorders and depression (33).

Different studies have proved that depressive and anxiety disorders are common among patients with headache (34-36). Some proved an association between musculoskeletal pain, arthritis and depression (10, 37). Studies in developed and developing countries showed that depression is two to three folds more likely among patients with arthritis as compared to patients without arthritis (9, 38-41)

Earlier researchers found an association between the numbers of chronic illness and depression (26, 30, 42). Our results proved no relationship between the actual number of chronic illnesses and depression. A proposed explanation that severity of the disease was not assessed with possible detrimental effect on quality of life and thus depression (43). Giving that patients who are disabled by multi morbidity are usually under the care of more specialized physicians and thus missed by our sample.

This study has its limitations as we used a screening tool to evaluate depression rather than diagnostic. Further studies are required to address the prevalence of depression in the primary care setting in a more diagnostic manner and its possible risk factors. A self –administered questionnaire is always uncertain and patients might under or overestimate their symptoms, the use of simple counts of the diseases may be by itself considered a limitation by others.

Conclusions

Depression is a common health problem encountered in the primary care setting. Many risk factors including age and some of chronic illnesses have been linked to the occurrence of depression. Primary care physicians are the patient's first contact and family doctors have to have a lower threshold in suspecting depression so they can identify those at risk and manage them when appropriate. Depression recognition and thus intervention can improve quality care provided to our patients, quality of life and even management of their comorbid medical illnesses. Further studies are needed to validate these findings in our local patient population.

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Atherosclerotic background of chronic obstructive pulmonary disease in sickle cell patients

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Abstract

Background: Metabolic syndrome is a systemic atherosclerotic process terminating with end-organ failures. We tried to understand presence of any atherosclerotic background of chronic obstructive pulmonary disease (COPD) in patients with sickle cell disease (SCD) in the present study.

Methods: All patients with SCD applying for any complaint were enrolled into the study. Cases with painful crisis and acute inflammatory events were treated at first, and then diagnostic tests were performed in a silent phase.

Results: The study included 256 patients with SCD (127 females). The mean age of them was 29.3 years. There were 15 (5.8%) patients with COPD with a highly significant male predominance (3.1% versus 8.5%, $p<0.001$). Digital clubbing and pulmonary hypertension were also higher in males, but the differences were nonsignificant in between (4.7% versus 6.2% and 11.0% versus 12.4%, respectively). Similarly, the leg ulcers were significantly higher in males, too (5.5% versus 16.2%, $p<0.001$). The significant male predominance was also observed in stroke and smoking (3.1% versus 6.2%, $p<0.05$ and 3.9% versus 11.6%, $p<0.001$, respectively). On the other hand, there were 13 (5.0%) mortal patients during the five-year follow-up period without any significant gender difference (5.5% in females and 4.6% in males, $p>0.05$), and the mean ages were 32.4 and 26.8 years, respectively ($p>0.05$).

Conclusion: SCD is an accelerated and systemic atherosclerotic process as in metabolic syndrome, and the higher prevalence of COPD in SCD patients may indicate that COPD may mainly be an accelerated atherosclerotic process of the pulmonary vasculature.

Key words: Atherosclerosis, metabolic syndrome, chronic obstructive pulmonary disease, sickle cell disease.

Introduction

Atherosclerosis may be the major health problem of the human being that decreases quality and duration of lifespan. Probably it is an irreversible process that accelerated by many factors. Although aging alone may be one of the unpreventable causes, smoking, dyslipidemia, obesity, diabetes mellitus (DM), hypertension (HT), and low and high-grade systemic inflammatory disorders are probably the accelerating causes of the systemic process. Such preventable causes of the systemic atherosclerotic process are mainly collected under the heading of metabolic syndrome (1-6). The syndrome is characterized by a group of metabolic risk factors including overweight, dyslipidemia, elevated blood pressure, insulin resistance, and a prothrombotic and proinflammatory state for the development of irreversible diseases such as obesity, HT, DM, coronary heart disease, peripheric artery disease, and stroke. Similary, chronic obstructive pulmonary disease (COPD) is also a frequent and continuously increasing cause of morbidity and mortality in the world (7). It is expected that COPD will be the third common cause of mortality and fifth common cause of morbidity all over the world by the year of 2020 (8). It is generally accepted that COPD is not solely a pulmonary disease instead it may just be one of the several consequences of a systemic process (9).

On the other hand, sickle cell disease (SCD) is a chronic hemolytic anemia that is characterized by sickle-shaped erythrocytes which is caused by homozygous inheritance of the hemoglobin S (Hb S). Polymerisation of the Hb S distorts erythrocyte into a sickle shape and decreases its elasticity. The abnormal shape and decreased elasticity cause a chronic endothelial inflammation terminating with an accelerated atherosclerotic process. We tried to understand presence of any atherosclerotic background of COPD in patients with SCD in the present study.

Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and June 2012. All patients with SCD applying for any complaint were enrolled into the study. Cases with SCD were diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography method. Their medical history including smoking habit, leg ulcers, and stroke was learnt, and a routine check up procedure including a computed tomography of the brain was performed. Current daily smokers for one pack-year and cases with a history of one pack-year were accepted as smokers. Cigar or pipe smokers were excluded. Clubbing was diagnosed by determining ratio of the distal phalangeal diameter to the interphalangeal diameter which is required to be >1.0 and with the presence of Swamroth sign (10,11). Cases with painful crisis and acute inflammatory events were treated at first, and then spirometric pulmonary function tests to diagnose COPD and a Doppler echocardiography to measure the systolic pulmonary artery pressure were performed in a silent phase. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) of less than 70% (8). Systolic pulmonary artery pressure at and above 40mmHg during the silent phase is accepted as pulmonary hypertension (12). Eventually, SCD patients with COPD, clubbing, pulmonary hypertension, leg ulcers, stroke, smoking, and exitus were detected and compared between the sexes. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 256 patients with SCD (127 females and 129 males). The mean age of them was 29.3 years (Table 1). There were 15 (5.8%) patients with COPD with a highly significant male predominance (3.1% versus 8.5%, $p<0.001$). Digital clubbing and pulmonary hypertension were also higher in males, but the differences were nonsignificant in between (4.7% versus 6.2% and 11.0% versus 12.4%, respectively). Similarly, the leg ulcers were significantly higher in males, too (5.5% versus 16.2%, $p<0.001$). The significant male predominance was also observed in stroke and smoking (3.1% versus 6.2%, $p<0.05$ and 3.9% versus 11.6%, $p<0.001$, respectively). On the other hand, there were 13 (5.0%) mortal patients during the five-year follow-up period without any significant difference between the sexes (5.5% in females and 4.6% in males, $p>0.05$), and the mean ages were 32.4 and 26.8 years, respectively ($p>0.05$) (Table 2).

Table 1. Characteristic features of the study cases

Variables	Mean age (year)	Female cases	Male cases	*p*-value
SCD*	29.3 ± 9.5 (14-59)	49.6% (127)	50.3% (129)	ns†
COPD‡	35.0 ± 8.7 (23-54)	3.1% (4)	8.5% (11)	<0.001
Clubbing	36.1 ± 12.1 (21-56)	4.7% (6)	6.2% (8)	ns
Pulmonary hypertension	30.4 ± 10.9 (19-56)	11.0% (14)	12.4% (16)	ns
Leg ulcers	35.7 ± 7.6 (17-58)	5.5% (7)	16.2% (21)	<0.001
Stroke	32.5 ± 9.2 (17-47)	3.1% (4)	6.2% (8)	<0.05
Smoking	33.1 ± 9.3 (21-54)	3.9% (5)	11.6% (15)	<0.001

**Sickle cell disease †Nonsignificant (p>0.05) ‡Chronic obstructive pulmonary disease*

Table 2. Features of the mortal patients

Variables	Female cases	Male cases	p-value
Prevalence	5.5% (7/127)	4.6% (6/129)	ns*
Mean age (year)	32.4 ± 10.6 (19-45)	26.8 ± 7.1 (19-39)	ns

**Nonsignificant (p>0.05)*

Discussion

COPD is an inflammatory disease that may mainly affect the pulmonary vasculature. The origin of the inflammation is unclear, but aging, smoking, and excess weight may be the major ones of the several possible causes. The inflammatory process is enhanced by the release of various chemical factors by lymphocytes to repair the damaged pulmonary tissues, especially endothelial cells of the pulmonary arteriols. However, due to the continuous irritation process of the endothelial cells in case of aging, smoking, or excess weight, prominent changes develop in the architecture of the airways and alveolar spaces, since the chronic inflammatory process of the endothelial cells terminates with fibrosis and atherosclerosis in the lungs. Probably the accelerated atheroscerotic process is the main structural background of the functional changes characteristic of the disease. Although COPD may mainly be an accelerated atherosclerotic process of the pulmonary vasculature, there are several reports about existence of an associated systemic endothelial inflammation (13-15). For instance, there may be a close relationship between COPD and CHD, peripheric artery disease, and stroke probably due to the systemic atherosclerotic process (16). In a multi-center study performed on 5,887 smokers aged between 35 and 60 years, two-third of mortality cases were caused by cardiovascular diseases and lung cancer, and CHD was the most common cardiovascular complication among them (17). When the hospitalizations were searched, the most common causes were the cardiovascular diseases again (17). In another study, 27% of all mortality cases were due to the cardiovascular causes in the moderate and severe COPD patients (18). Similarly, beside the clubbing, pulmonary hypertension, leg ulcers, and stroke like atherosclerotic end-points, COPD is just one of the final consequences of the SCD, as an accelerated atherosclerotic process, in the present study.

Aging, smoking, and excess weight are probably the most significant causes of the systemic atherosclerosis. Adipose tissue may function as an endocrine organ, and causes a systemic inflammatory reaction. The systemic inflammatory effect of smoking on endothelial cells is already known with Buerger's disease. Increased oxidative stresses, inactivation of antiproteases, and release of proinflammatory mediators may terminate with a systemic inflammatory process in smokers. The systemic inflammatory process particularly affects the pulmonary endothelial cells due to the higher concentrations of the irritant substances of smoke in the pulmonary vasculature. So COPD may actually be a kind of Buerger's disease mainly affecting the pulmonary vasculature. Prominent leg involvement of Buerger's disease may also be secondary to the higher concentrations of the irritants of smoke in the leg vasculature due to gravity. Similarly, aging may be another but unpreventable cause of systemic atherosclerotic process that prevents adequate tissue repair. The prevented adequate tissue repair due to the systemic atherosclerotic process may be a significant cause of the increased risk of cancers in elders. Since, immune cells can not eradicate the malignant ones effectively due to the prevented adequate tissue circulation in them. On the other hand, both the COPD frequency and its complications are increasing in the society. For example, although age-matched mortality for all other diseases decreased with a 32% ratio in the last 30 years, the COPD related mortality increased with a 102% ratio in the same period of time (19). According to the most optimistic estimates, the COPD mortality rates will increase by 50% over the next 15 years (20). Although the achieved development in the health services and decreased smoking prevalence worldwide, the increased COPD mortality and morbidity may only be explained by aging of the society and increased frequency of excess weight in the world. Similarly, the mean age, mean pack-years, and mean BMI increased progressively from the mild towards the severe COPD cases in another study ($p<0.05$ nearly in all steps) (21). The only exception was the decreased mean BMI after the moderate COPD cases, probably due to the severe COPD induced cachexia in the body. The overall male predominance of the COPD, leg ulcers, and stroke cases in the present study may also indicate the strong atherosclerotic effects of smoking, since the smoking is significantly higher in males all over the world (21).

Hb S causes erythrocytes to change their elastic biconcave disc shape to a hard sickle shape especially during mild, moderate, and severe stre-

sses, but the sickling is usually seen during the whole periods of life. The erythrocytes can take their normal elastic shapes later, but after repeated cycles of sickling and unsickling attacks, they get a permanent sickle shape with a loss of elastic motion ability that is especially important during the passage between the endothelial cells. So they cause damage on the vascular endothelial cells terminating with a chronic endothelial inflammation. Because of the lifelong duration of the chronic endothelial inflammation, an accelerated atherosclerotic process develops all over the body. Although the chronic inflammatory process is exaggerated during infections, operations, or depressions like various stresses, it is usually present during the whole lives of the patients. The chronic process is usually shown by a permanent leukocytosis and thrombocytosis even in silent phases of the patients. The adverse effects of neutrophils on endothelium are of particular interest with regard to CHD and stroke in SCD. For example, leukocytosis during the silent phase was an independent predictor of the severity of the disease in a previous study (22), and it was associated with the risk of stroke in another study, too (23). On the other hand, due to the accelerated atherosclerotic process, SCD may be a useful model to show the end results of systemic atherosclerosis seen with the metabolic syndrome even in early age groups (24). The very high prevalences of COPD (5.8%), digital clubbing (5.4%), pulmonary hypertension (11.7%), leg ulcers (10.9%), stroke (4.6%), and exitus (5.0%) even in the early age group (29.3 years) may be a good sample to show some end results of the systemic atherosclerosis in the present study.

As a conclusion, the metabolic syndrome is a systemic atherosclerotic process terminating with end-organ failures. SCD is an accelerated and systemic atherosclerotic process, and the higher prevalence of COPD in SCD patients may indicate that COPD may mainly be an accelerated atherosclerotic process of the pulmonary vasculature.

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Effect of attachment behaviors education on level of maternal-fetal attachment

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Abstract

Introduction and objective: Maternal-fetal attachment shows the relationship between a pregnant woman and her fetus, and indicates the emotional relationship between them. Some interventions can foster the relationship between a mother and her unborn baby. Developing the maternal-fetal attachment can build a healthy relationship between a mother and her infant, and promotes cognitive, emotional, and social growth. Based on this, the present research is done to determine the influence of attachment behaviors training on the level of maternal-fetal attachment among first-time pregnant mothers.

Materials and methods: This is an interventional research conducted on 83 eligible pregnant mothers. These mothers were selected using sampling method, based on research's objective, from those pregnant mothers who visited health centers. They, then, divided randomly into case and control groups. The instruments used for this study include maternal-fetal attachment questionnaire and interview form. Four 2-hour attachment behavior-training sessions were hold for case group (a session per week). During that, attachment behavior forms were distributed among mothers to complete, in a weekly manner. The control group received the routine pregnancy cares. Attachment scores were measured before and after intervention in both groups. To analyze the data, chi-square, Fisher's, Mann-Whitney, and t- tests were applied.

Findings: Findings showed that the average scores of maternal-fetal attachment were 3.52 ± 0.5 and 3.54 ± 0.43 in case and control groups, respectively, which statistically indicated that there was no meaningful difference ($p = 0.78$). However, these average scores were, in turn, 3.96 ± 0.38 and 3.42 ± 0.41 after intervention, showing meaningful statistical difference ($p<0.001$).

Conclusion: The finding shows that training and doing some attachment behavior can increase the level of maternal-fetal attachment, which per se creates appropriate relationship between the mother and her infant, and promotes cognitive, emotional, and social growth of the baby.

Key words: Education, maternal-fetal attachment behaviors, first-time pregnancy.

Introduction

Maternal-fetal attachment is a term that describes the relationship between mother and her foetus. Emotional attachment towards unborn child starts from the first days of pregnancy and this feeling of love, interest, and attachment reaches its peak during second trimester (1). Maternal-fetal attachment is formed based on mental visions a mother has of her baby (2, 3). It can include mental vision of the relationship between mother and child in a way that the mother attributes specific physical and emotional characteristics to the fetus, and appears in such behaviors, which are indicative of mother's attention and care in her fetus. Having good diet, avoiding harmful materials such as alcohol, having optimistic visions of the fetus, talking with fetus, paying attention to fetal movements, and so on are some examples of such behaviors (3, 4). The mothers with higher level of attention would have more interactions with their infant that per se can significantly affect emotional, cognitive, and social development of the child(5). On the other hand, low level of attachment may be connected to different type of fetal mistreatment. This is possible that the mothers who smoke more, drink alcohol, and do less healthy behaviors, have less attachment with their fetuses (6). Kelly writes, "Those mothers who have less attachment to fetus are reported to show less healthy behaviors" (7). Researches show that some interventions strengthen the bond between mother and her unborn child (4).

Pregnant mothers tend to healthy behaviors, especially when they believe these behaviors promote the health of their fetuses (8). The Kelly's study shows that maternal-fetal attachment has positive relationship with healthy behaviors (7). Among the ways that cause more healthy behaviors is to train these behaviors. Training is the foundation of all healthy behaviors and has significant role in changing people's thoughts, behaviors, and habits (9). There are several evidences

showing that specific trainings are related to healthy behaviors, in such a way that trained people display more healthy behaviors (10). World Health Organization regards training as the key component of prenatal care (11). Bellieni *et al*. showed in their study that training during pregnancy can has positive influence on maternal-fetal attachment (12). In addition, Magdi in a research has stated that counting fetal movement by mother promotes maternal-fetal attachment (13). Therefore, we can pave the ground for mothers to show these behaviors by training them with maternal-fetal attachment behaviors. Regarding the few number of relevant studies, this research has been conducted with the aim of determining the influence of training attachment behaviors on the level of maternal-fetal attachment among first-time pregnant mothers who visited health centers in the Sari County.

Research method

The present study is an interventional research with a sample group comprising 83 eligible pregnant mothers who visited health centers in Sari County. Regarding eligibility, the qualified samples were between 18 and 35 years, finished secondary schools, experienced pregnancy for the first time, were in the 28-32th week of pregnancy, did not have any problem relevant to midwifery, and did not have mental problem history. The sampling was objective-based, in which three centers were assigned for control group and three centers were allocated for case group. In order to determine the sample size, study guide and sample size formula ($n = \frac{(u+V)^2(S_1^2+S_2^2)}{(m_2-m_1)^2}$) have been applied. A sample size of 50 was considered for each group among them 10 people from case group and 7 people from control group were excluded during the study. Therefore, the final analysis was done on 40 samples of case group and 43 samples of control group.

The instruments applied in this research have included interview form and Cranley's maternal-fetal attachment scale (MFAS). In order to determine the validity of interview form and maternal-fetal attachment questionnaire, content validity method was applied. Reliability of interview form and that of maternal-fetal attachment scale were confirmed using test-retest and Cronbach's formula (80%), in turn. Maternal-fetus attachment questionnaire includes 24 items scored from 1 to 5 (definitely yes-5, yes-4, not sure-3, no-2, definitely no-1). Only in item 22 the scoring is reversed, i.e. (definitely yes-1, yes-2, not sure-3, no-4, definitely no-5). The average score is obtained through dividing all the scores by the number of items. Four 2-hour attachment behavior-training sessions were hold for case group (a session per week in one month). In the first session, concepts of maternal-fetal attachment, behaviors related to maternal-fetal attachment, advantages of attachment, and the way attachment behaviors should be done were taught. These behaviors had included counting and recording the number of fetal movement, having optimistic vision of appearance of fetus, picturing themselves feeding their babies, and abdominal palpation. In addition, the relevant forms for recording desired behaviors were distributed to the mothers in a weekly manner to be completed after doing those behaviors. In the next sessions, the mothers revisited health centers to give back their behavior recording forms to be checked, and they were asked to exhibit some of desired behaviors. The researcher and other members of the group helped the mothers to correct their behaviors, if necessary, and then they were asked to complete the forms. No intervention was made in control group and routine care was delivered. Mothers completed the maternal-fetal questionnaires before and after intervention. Data analysis was done using SPSS 11. To express the properties of research samples, descriptive statistics including frequency tables, mean, and standard deviation were used. In order to investigate the homogeneity of two groups, in terms of quality variables, chi-square and Fisher's tests were applied. For com-

paring two groups, independent t-test was used in different stages and to compare each group, paired t-test were applied in two different stages. In all tests, confidence coefficient of 95% and meaningful level of 0.05 have been considered.

Findings

The findings show that average age of research samples in case group was 24.13 ± 3.7, and 24.1 ± 4.4 in control group. Two groups are homogeneous in terms of age, education, socio-economic status, accommodation situation, income mean, interest in the partner, and marital satisfaction (table 1). The mean gestational ages in case and control groups were 29.6 ± 1.5 and 29.5 ± 1.6, respectively. The people under study were homogeneous in both groups regarding planned pregnancy, going for sonography test, prediction of fetal sex, and predicted sex (table 1). The mean of attachment scores have been 3.52 ± 0.5 and 3.96 ± 0.38 in case group, and 3.54 ± 0.43 and 3.42 ± 0.41 in control group, before and after intervention. In the beginning of study, the mean of attachment score had no meaningful statistic difference in two groups, based on t-test ($p = 0.15$). However, after the study, this difference were meaningful ($p < 0.001$). In addition, there was no meaningful difference in the mean of attachment score in control group, in the beginning and at the end of study, based on paired t-test; while, this

Table 1. Study of case and control groups homogeneity based on some variables

Variable studied in both groups	p-value and statistical test	
Age	$p = 0.96$	$T = 0.44$
Education	$p = 0.25$	$X^2 = 1.27$
Socio-economic status	$p = 0.77$	$X^2 = 0.5$
accommodation situation	$p = 0.84$	$X^2 = 0.039$
Interest in the partner	$p = 0.55$	$Z = 0.58$
marital satisfaction	$p = 0.75$	$Z = 0.31$
planned pregnancy	$p = 0.88$	$X^2 = 0.02$
sonography test	$p = 0.17$	$X^2 = 1.87$
Fetal sex prediction	$p = 0.24$	$X^2 = 1.36$
Predicted sex	$p = 0.47$	$X^2 = 0.51$
Income mean	$p = 0.84$	$Z = 0.51$

Table 2. Comparing the mean of maternal-fetal attachment score before and after intervention, and the difference before and after, according to each group separately

Time	Before study	After study	Difference between before and after	Paired t
Statistical index	m ± SD	m ± SD	m ± SD	
Group				
Case	3.52 ± 0.5	3.96 ± 0.38	0.44 ± 0.28	t = 10.0 p < 0.001
Control	3.45 ± 0.43	3.42 ± 0.41	-0.028 ± 0.13	t = 1.4 p = 0.14
Independent t	t = 0.65 p = 0.512	t = 6.1 p < 0.001	t = 9.7 p < 0.001	

Table 3. Linear correlation between maternal-fetal attachment and social support, mother's education, interest in the partner, and prediction of fetal sex

Statistical index Variables	r	p-value
Social support	0.85	$p < 0.001$
Mother's education	0.59	$p < 0.001$
Interest in the partner	0.26	$p = 0.015$
Prediction of fetal sex	0.29	$p = 0.007$

difference was meaningful in case group. (table 2). In order to look into the relation of some variables with maternal-fetal attachment, Pearson's correlation was used. The results are presented in table 3.

Discussion

The prenatal cares are good opportunities to assess maternal-fetal attachment, and to make some interventions to promote that attachment. In order to evaluate the level of attachment, the mean of overall attachment score of research samples was 3.48. Koniak in a similar study reported the level of attachment as 3.8 in case group and 4.33 in control group, before intervention (14). Kelly *et al*. reported that the level of maternal attachment was 3.77 (7). The average age of pregnancy in their research was 29 weeks. These two studies show that the level of maternal-fetal attachment in their research samples was more than that of in the present study. This difference can be attributed to the differences in research community, culture, and facilities. In this study, a meaningful correlation between social support and maternal-fetal attachment was seen. In Koniak's study, no meaningful correlation between attachment and social support was found in pregnant adolescents. However, Condon and Corkindal, in a similar study, found a meaningful correlation between social support and attachment in pregnant non-adolescents (15). Cranley has also reported a positive correlation between those two variables (16). In addition, a meaningful correlation was found between mother's education and maternal-fetal attachment. Regarding that, the result obtained from Kwon and Bang's research has shown that mothers with lower level of education have exhibited less attachment (17). However, Cannella in a review study has stated that ''Willson, Kemp, Curry, and Grace have found the lack of correlation between those two variables" (18). In this study, it is shown that there is a correlation between maternal-fetal attachment and marital satisfaction .Blooms research showed that there is positive relation between satisfying relationship with the father and MFA(19). In addition, the level of attachment, after intervention in two groups, showed meaningful difference. Moreover, the mean of difference in two groups, before and after maternal-fetal intervention, was meaningful. In Koniak's study, the difference before and after attachment intervention in two groups has been meaningful (14). In the research done by Magdi *et al*., the level of attachment, after intervention (counting fetal movements by mother) in case group has also been meaningful (13). In addition, in a research conducted by Bellieni *et al*., the group received training (four 2-hour sessions) showed higher attachment score than control group (12). Chang *et al*., also, founded in their study that holding prenatal classes (two hours per week for four weeks) to train maternal-fetal interactive behaviors, breathing techniques, and certain exercises to be done by mothers can increase the level of maternal-fetal attachment (20). In another study, it is shown that programs designed to promote maternal-fetal interactions, such as talking to and feeling the baby via touching the abdomen by first-time pregnant mothers, would boost maternal-fetal attachment (21). However, Carson in Washington University has stated that there is no meaningful relation between having massage and feeling the baby by touching abdomen, and maternal behaviors after delivery (22). In most of these studies, as well as the present research, the level of attachment has been increased after intervention. As Magdi stated, "this increase can be related to behaviors such as counting fetal movements and mother's positive vision of her baby which involve her in activities that stimulate her emotions and interaction with the fetus" (13). Interventions aiming at enhancing maternal sensitivity to fetal activities, such as fetal movement, can increase the level of maternal-fetal bond (21). Mothers, who make these interventions, more would show further maternal behaviors, which per se can affect their health. On the other hand, maternal-fetal attachment can be a good predictor for primary relation between mother and her infant. Increasing attachment during pregnancy can create more confidence in doing maternal role, and so mother would be adjusted better with conditions developed after delivery of the baby and show positive reaction to infant's behavior (5).

Conclusion

The research's result shows that training behaviors related to attachment increases the level of maternal-fetal attachment. In that case, mother forms attachment to her child, the child becomes important for mother, and she would be motivated to meet the

child's demands. Therefore, pregnant mother who feels attachment to her fetus attempts to ensure the health and comfort of her child by taking care of herself. Therefore, in prenatal visits to health-care centers or clinics, simple attachment behavior trainings can be used to improve the relationship between mother and her infant and foster the social, emotional, and cognitive growth of child, and to begin improving the maternal-fetal bond from fetal period.

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An evaluation of the embryotoxicity of titanium miniplates using the chicken embryotoxicity screening test

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Abstract

The aim of this study was to evaluate the embryotoxicity of titanium miniplates in hens using the embryotoxicity method. A total of 370 hen eggs were separated into 10 groups: the control group (C1), the pierced and sealed group (C2), 30% ethyl alcohol (ETOH)-injected group, 20 ng aflatoxin B1 (AFB1)-injected group, 0.1 M phosphated 7.4 pH isotonic solution (PBS; denoted "P")-injected group, and phosphate buffered saline (PBS) including 3% bovine serum albumin (PBS+BSA; denoted "P+B")-injected group. The remaining four groups were treated with commercially available miniplates. Two of the miniplates were fixed to bovine bone to construct a deformed miniplate (II). The other two were not fixed (0). The fixed plates were removed and all were treated separately with two different washing solutions consisting of P and P+B. After analysis on the 7th and 17th days of incubation, embryo growth stages were assessed according to the Hamburger-Hamilton scale. In the assessment of embryonic deaths, only eggs with embryos were included. Mortality rates were significantly higher in miniplate groups than in the control group ($P < 0.05$). In addition, differences between the AFB1 group and the miniplate groups were statistically significant.

Key words: Miniplate, CHEST, AFB1, metal ion release, embryotoxicity.

Introduction

Fixation methods with titanium miniplates have been used frequently in the reconstruction of maxillofacial neoplastic lesions and fractures [1]. Immobilizing fragments with wires is also a technique in the management of facial fractures. However, wiring techniques are inadequate for sufficient stabilization in most cases and should be supported with other methods such as intermaxillary fixation (IMF) [2]. Using the IMF method alone (except in certain cases) is currently a rare approach due to some drawbacks that became apparent recently [3]. Using miniplate rigid fixation with or without IMF can also be performed in the management of maxillofacial fractures. Titanium miniplate use is a common approach due to low morbidity rates and painless early mobilization, whilst at the same time providing stability. Alloys or "pure" implanted materials are potential sources of toxic metal ions and particles. Many metal plates and screws recently used in orthopaedics and traumatology have been observed to release ions into surrounding tissues [4]. Several investigations have reviewed the potential biological effects of released metals [5- 7]. Furthermore, studies have confirmed a significant increase in the concentrations of Co, Cr, Ni, Mo, and Ti within surrounding soft tissues 6 months after initial miniplate insertion into the mandible [8-10]. For suitable materials to be implanted into tissues, sufficient biocompatibility has to be demonstrated due to possible ion release and direct toxic effects on neighbouring tissues.

The primary biomaterial used for osteosynthesis in maxillofacial surgery is titanium. Alloys or pure titanium are also frequently used to fix fractures in maxillofacial regions [11]. Titanium is known to be highly biocompatible and resistant to corrosion [7]. Despite recent evaluations performed by different methods have not shown an evident toxicity of nanoparticles of the titanium alloy, some studies have reported local inflamma-

tory effects of nanoparticles to the adcajent tissues [4, 7, 12, 13]. However titanium miniplates' teratogenic and embryotoxic effects have not been evaluated yet for mammals.

The aim of this study is to evaluate the embrotoxic effects of the titanium miniplates by the chicken embryotoxicity screening test (CHEST).

The CHEST in which impregnated chicken eggs are used, has been developed to determine embryotoxic and teratogenic effects of several chemicals and metals [14]. In order to determine the embryotoxicity of miniplates used in maxillofacial surgery, we applied the CHEST.

Materials and methods

This study was performed using 370 eggs derived from Nick Chick broods.

Miniplates

We used commercially available, 1 mm thick, 4-holed 4 titanium alloy miniplates (Walter Lorenz Inc., Florida, USA - Grade 2 – product code: M-01-9204). The contents of the titanium alloy were 0.03 % Nitrogen, 0.1 % Carbon, 0.125 % Hydrogen, 0.3 % Iron, 0.25 % Oxygen and 99.45 % Tinanium.

The surface areas of the miniplates were calculated using the Net CAD Mapping Program (Ak Engineering, Ankara, Turkey). The mean surface area value was 194.684 mm^2.

Miniplate preparation

Two of the four miniplates were adapted and screwed onto a bovine rib that could act as a human bone with its structure to achieve used miniplates and subsequently removed. The used miniplates had been crooked and reshaped onto bovinebones natural surface. The remaining two miniplates were left untreated. Used miniplates were washed with a soft toothbrush under isotonic irrigation. Thereafter, miniplates were brushed with a 95% ethanol solution and left to dry overnight. The following day, miniplates were sterilized by dry air sterilization (160 C^o, 2 hours) and placed in tubes. At the same time, a 0.1 M phosphated 7.4 pH isotonic solution (P) and 0.1 M 7.2 pH phosphate buffered saline (PBS) including 3% bovine serum albumin (P+B) were prepared. The P and P+B solutions (15 mL respectively) were placed into two tubes, each containing both used and unused plates, until the entire surface of the plate was covered. Thereafter, tubes were shaken using a shaker device (Gerhardt SW 20, Germany).

Ti and Fe ions in tested solutions

Ti and Fe ion concentrations were analysed using an inductively coupled plasma atomic emission spectrometer varian-vista, Australia pty Ltd (ICP-AES; 14Skujins, 1998) in order to determine whether the ions passed through the P and P+B solutions during the shaking period.

Aflatoxin preparation

Aflatoxin B1 (AFB1), a well-known embryotoxin, was used as a positive control. Standard crystallized pure AFB1 (Makor Chemical Co, Israel) was dissolved in benzene and AFB1 solution was prepared for positive control group. The solution was then transferred to sterile tubes and benzene evaporation was performed. The AFB1 inside the tubes was completely dissolved by adding 99.9% ethylene alcohol (ETOH). Thereafter, to decrease the concentration of ETOH to 30%, distilled water (20 µg AFB1/20 ml) was added to allow the solution to be used for the "positive" control group. The prepared solution was tested to analiyze the concentration of AFB1 by using thin layer chromatograph techniques (TCL) and a fluorescence spectrophotometer that was an electromagnetic spectroscopy analysis method (Perkin Emler MPF 43-A; emission 425 nm, excitation 365 nm) using standard tablets (Merck) wrapped in aluminium foil and maintained at a room temperature of +4°C.

Experimental groups and solution injection into eggs

In this study, eggs were separated into 10 groups, each with 37 eggs. The groups, abreviations of groups and procedural tests are summarized in Table 1. Before injection, all eggs were weighed and disinfected indoors (25 C^0 room temperature) for 20 minutes by subjecting them to a gas consisting of 80-gram potassium permanganate added to 130 ml of 40% formaldehyde per cubic meter. Holes were made on the eggs by a drill for all injections. Injections were made through a hole using micropipettes (Sealpette, Jencons, Finland). In all groups, 20 µl of the test

solution was injected to air cell of the egg. Holes were then sealed with paraffin (Merck, 56-58˚C melting point). Incubation took place under optimal conditions (37.8˚C heat, 65% moisture) using a 1200 egg capacity incubator (Gostyn, Poland) in the Department of Histology and Embryology, Veterinary College, Selcuk University.

To analyze if the embryos were dead or live, eggs were exposed by cutting the eggshells by a tissue scissor on the 7th and the 17th day of incubation on which of the periods heart beat and respiratuar system was established respectively. Embryos were removed, washed in distilled water, weighed on the 7th and the 17th day and relative embryo weights were calculated ([Embryo weight / egg weight before the injection] x 100). The developmental period was determined using the Hamburger-Hamilton scale (9). After both the 7th and the 17th day of incubation, all embryos were observed with the naked eye, data recorded, and images captured. All samples were then moved to a 10% formaldehyde environment.

Statistical analysis

To determine embryonic death rates, a YATES Chi-Square test was applied. In order to compare parameters among groups a Tukey HSD post hoc test was performed. SPSS 10.0 (SPSS Inc., Chicago) was used for all statistical analyses. The significance level (P) was set at $P < 0.05$.

Results

Amounts of Ti and Fe ions those were dissolved components from the plates in solution were measured using an ICP-AES device (Varian-Vista Australia Pty Ltd.; Table 2).

Embryonic death rates and macroscopic findings

Out of 370 eggs, a total of 345 were impregnated. The mean "non-impregnated egg ratio" was 6.76 %. With the exception of infertile eggs, only embryo-containing eggs were used for evaluations of embryo development on the 7th and 17th days, and death rates were calculated. The mortality rate of the control group was 0%, whereas the drilled and immediately closed group showed a mortality rate of 8.33% (Table 3, Figure 1). When 30% ETOH was used, the AFB1 group demonstrated a significantly higher mortality rate (20%) than the control group ($P < 0.05$). A similar result was observed for the 20 ng dose AFB1 eggs, with 73% higher mortality rate than in the control group ($P<0.05$). The titanium miniplate groups also showed significantly higher mortality rates than the control group.

Table 1. Experimental groups with abreviations and applied procedures

Groups (n=37)	Applied procedures
1. Control group (C1)	None
2. Drilled and Sealed group (C2)	Exposed and sealed with liquid paraffin
3. 30 % ETOH group (ETOH)	30 % ETOH injection
4. AFB1 group (AFB1)	20 ng AFB1 per egg AFB1 injection
5. PBS group (P)	PBS shaking solution injection
6. PBS+BSA group (P+B)	PBS+BSA shaking solution injection
7. Lorenz O* PBS+BSA group (L O P+B)	P+B solution shaken with unused Lorenz miniplate
8. Lorenz O* PBS group (L O P)	P solution shaken with unused Lorenz miniplate
9. Lorenz II* PBS+BSA group (L O P+B)	P+B solution shaken with used Lorenz miniplate
10.Lorenz II* PBS group (L O P)	P solution shaken with used Lorenz miniplate

O*= unused miniplate, II*= used miniplate

Table 2. ICP element analysis results of shaken liquids used as test solutions (ppm)

Groups	Ti mg/L=ppm	Fe mg/L=ppm
Lorenz O P+B	0.020	0.104
Lorenz O P	0.030	0.025
Lorenz II P+B	0.028	0.097
Lorenz II P	0.032	0.017

The relative embryo weights of the control group at 7th day was found to be 0.72±0.07 gr. The relative embryonic weights of the AFB1 group were found to be statistically less as compared with the control group (C1) (0.46±0.07). However there was not a statistically difference between the miniplate groups and C1 group. Regarding to the embryonic weights at 17th day of the incubation period, the embryonic weights of the control group was found to be 20.62±2.26. AFB1 group was found to be less than the C1 group (17.68±2.64) (Table 4). However, miniplate groups were not found to be statistically different than the control group.

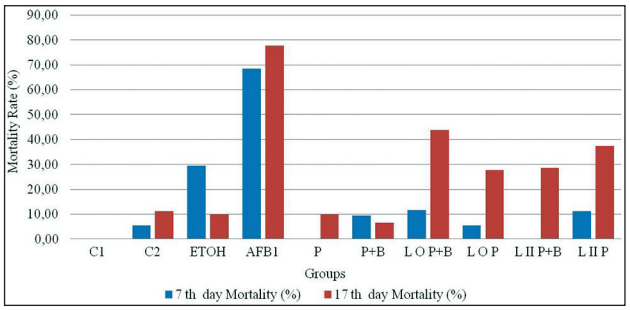

Figure 1. Embryonic death rates within groups according to the Hamburger-Hamilton scale

Table 3. Embryonic development observations made on the 7th and 17th days

Groups	Incubation Periods					
	7 th day			17 th day		
	Live number	Dead number	Mortality (%)	Live number	Dead number	Mortality (%)
Control group (C1)	17	0	0	15	0	0
Drilled and sealed group (C2)	17	1	5,56	16	2	11,11
30% ETOH group (ETOH)	12	5	29,41	16	2	10
AFB1 group (AFB1)	6	13	68,42	4	14	77,78
PBS group (P)	18	0	0	16	2	10
PBS+BSA group (P+B)	19	2	9,52	14	1	6,67
Lorenz O* PBS+BSA group (L O P+B)	15	2	11,76	9	7	43,75
Lorenz O* PBS group (L O P)	17	1	5,56	13	5	27,78
Lorenz II* PBS+BSA group (L II P+B)	17	0	0	10	4	28,57
Lorenz II* PBS group (L II P)	16	2	11,11	10	6	37,5

O= unused II*= used*

1= only embryo consisting egg numbers were evaluated (except infertile).

Table 4. Results of statistical analyses for numbers of embryos (except infertile) and relative embryo weights on the 7th and 17th days of incubation

Groups	Incubation periods			
	7 th day (X±SE)		17th day (X±SE)	
	N gr.		N gr.	
Control group (C1)	17	0.72±0.07^{ef}	15	20.62±2.26^{bcdef}
Drilled and sealed group (C2)	18	0.60±0.09^{bcdef}	18	19.24±1.46^{abcdef}
30% ETOH group (ETOH)	17	0.63±0.07^{cdef}	18	17.78±1.64^{abc}
AFB1 group (AFB1)	19	0.46±0.07^{ab}	18	17.68±2.64^{ab}
PBS group (P)	18	0.45±0.14^{a}	18	16.13±1.86^{a}
PBS+BSA group (P+B)	21	0.52±0.11^{abc}	15	17.90±2.28^{abcd}
Lorenz O PBS+BSA group (L O P+B)	17	0.70±0.10^{ef}	16	22.20±2.94^{ef}
Lorenz O PBS group (L O P)	18	0.72±0.07^{ef}	18	22.01±1.64^{ef}
Lorenz II PBS+BSA group (L II P+B)	17	0.69±0.11^{def}	14	22.69±2.81^{f}
Lorenz II PBS group (L II P)	18	0.58±0.12^{abcdef}	16	21.55±2.62^{def}

1. No difference was found between means that named with same letter on same column according to Tukey HSD test (p>0.05)

Statistical analysis of control and experimental groups

Statistical comparisons of dead versus live embryos in all groups are shown in Table 3. There was no significant difference between the number of dead versus live embryos between used and unused miniplates ($P > 0.05$). There was, however, a statistically significant difference between AFB1 groups and miniplate groups ($P < 0.05$; Table 3).

Discussion

Titanium miniplates are preferred osteosynthesis materials, as they possess many advantages. Some metal remnants of miniplates that are used for rigid fixation in maxillofacial fractures remain inside adjacent soft and hard tissues. It is possible that some remnants may have occurred during placement of the plates; metal remnants can be left behind as a result of friction between plates and screws with other instruments. In addition, attrition and corrosion in plate-screw gaps create metal remnants of fragmentation products [15]. Of particular concern is the fact that corrosion leading to the deterioration of metallic implants can release by-products that are not biocompatible with the human body [12]. In our knowledge embryotoxicity of the titanium miniplates has not been evaluated yet in the literature. The present study aimed to correct this issue by the CHEST method.

In this study, 2 of 4 commercially available miniplates were screwed into and removed from the bovine rib. This procedure was successful in causing microfractures and attritional corrosion similar to that which arises during placement within the human body.

Winged embryos are the preferred specimens for analysing the genotoxic and teratogenic effects of medications, chemical materials, and aflatoxins [16]. To achieve this goal, Jelinek established a chicken embryotoxicity determination test for impregnated eggs (CHEST) [17]. This test can be performed easily in a short time and is cost effective. In the present 370 eggs were used. The number of the eggs was confined by the labaratory conditions.

In this study, control group injections were performed before incubation to assess the embryotoxicity of the non-metabolized native form of AFB1. Test solution injections were also made prior to incubation, as it is well known that titanium is not metabolized.

Previous studies have shown that mortality because of embryotoxicity were usually seen on the 7th and the 8th days of the incubation period. In addition Mauldin and Buhr reported that lung respiration was seen on approximately 17th 18th days Therefore embryos were analyzed on the 7th and the 17th days on which of the periods heart beat and respiratuar system was established respectively.

The results pertaining to embryonic deaths (according to the Hamburger-Hamilton scale), as determined by injecting AFB1 within this period, were similar to the results obtained by other investigators [18]. Verret et al. reported that AFB1 injections before incubation cause maximum mortality, and deaths are intensified after the first 8 days [19]. On the other hand, in the current study, we observed an increase in death frequency after the first 7 days. The 52nd hour (17th phase of Hamburger-Hamilton scale) after incubation is critical for development as it is the point at which a regular heart beat is established and the embryo is very sensitive to environmental stimuli during this time [19]. An increased prevalence of deaths within the first 7 days may be explained due to mitotic activity and differentiation of cells, as well as the increased sensitivity of the embryo to physical and chemical stimulants. An additional stage of increased sensitivity was found on the 17th day of incubation during which the chick pierces the internal membrane within the shell switch from allantoises respiration to lung breathing. This information may explain the cause of increased deaths on day 17. In the current study, we found 154 live and 26 dead embryos on day 7. In addition, we found 123 live and 43 dead embryos on day 17.

Many authors have concentrated their efforts on the development of CHEST in terms of optimizing the injection area within eggs (the method of injection) and the age of the embryo (injection session) [20, 21]. Opinions vary as to the reliability of injection methods with regards to the particular substances being tested. Prelusky et al. reported four different injection areas of AFB1 (to air cell, to the albumen at a 45^0 angle, to the equator region, and to the yolk part of the egg) and they found no significant differences between the regions in terms of AFB1 toxicity.

On the other hand, Verret et al. emphasized that injection via the yolk had less toxic effects as compared with the air cell approach [19]. They also found that the OD_{50} value of yolk-injection was 48 ng/egg, while the air cell method led to a lower value of 25 ng/egg. On the other hand, many investigators prefer the air cell injection method for its ease of use and leak-free benefits, better sterilization conditions, fast and homogenous solution diffusion, and elimination of internal pressure that can cause mechanical damage to an embryo [22, 23]. It is for these reasons we choose the air cell injection method.

The type of solvent, volume and concentration of solution, dose, and number of eggs per dose are important factors for *in vivo* embryotoxicity tests. AFB1 is preferred to ETOH [24]. Nevertheless, propylene glycol and chloroform have also used as solvents [19, 24]. It is commonly agreed upon that the ideal concentration of solvent is 30% prepared with sterile distilled water. An application of 20 µl 30% ETOH has a 20.8% mortality ratio, whereas the same volume of 95% ETOH has a much higher (43.1%) mortality ratio. Different studies recommend a volume test that includes volumes between 20-100 µl [25]. However a 20 µl volume is reported as being ideal for air cell injections [26]. Prelusky et al., also showed a 20.8% mortality rate with 30% ETOH using a volume of 20 µl and a 59.7% mortality rate with the same solution at a volume of 100 µl [27]. In this study, the mortality rate was found to be 20% for 20 µl 30% ETOH. This value is very close to the one that was found reported by Prelusky. In particular, the mortality rates of three of the miniplate groups (LOP+B, LOP, LIIP) were statistically significant as compared with control ($p < 0.05$). Although, there was no statistically significant difference between the mortality rate of the positive control group (AFB1) and the mortality rate of miniplate groups (LOP+B, LOP, LIIP), it is clear that the AFB1 group had higher mortality rates.

In this study, the mortality rate of the control group was 0%, while the pierced and sealed group (C2) had a mortality rate of 8.33%. Neither the C2 group nor the shaken solutions injected group (P and P+B) were significantly different from the control group ($p > 0.05$). This indicates that the C2 procedure and shaken solutions have little toxic effects.

Relative embryo weigths were calculated to consider the loosed weights through evaporative moistre loss. Harvey reported that evaporative moistre loss affected the 15% of the original weight [28]. Relative embryo weights were significantly less in AFB1 injected groups as compared to the control group (C1) at 7th and 17th days. Since the other groups were found to be less toxic, there were no differences when comparing the relative embryo weights between the other groups and control group. It might be thought that high embryo toxicity such as in the AFB1 injected group effected the embryonic development negatively.

Rosenberg has shown the existence of up to 5 µms of extended metal particles caused by microfractures during plate adaptation [27]. Langford et al. excised soft tissue specimens from the tissues adjacent to titanium miniplates which had been in situ for between 1 month and 13 years and identified metal debris between 1 to 200 µm lying within fibrous connective tissues [29]. Particles of titanium in the tissues have been shown to active monocytes and macrophages, to release the bone - resorbing mediators and to stimulate fibroblasts to increase collogen synthesis [29, 30]. Plate damage secondary to either surgical manipulation whilst in situ has been suggested as an important cause of titanium release [31]. Black pigmentation is frequently encountered in adjacent tissues during the removal of miniplates. This pigmentation has been defined as an accumulation of metal particles known as "metallosis". Some authors have macroscopically observed as high as 25.6% black pigmentation in the tissues surrounding titanium miniplates, while observing no pigmentation on the removal of Champy stainless steel plates [27]. Matthew and Frame placed Champy stainless steel and titanium miniplates on the frontal bone of animal models [32]. Thereafter, they found metal particles in tissues surrounding the plates. However, they did not investigate the effects of titanium miniplates on adjacent tissues. Although Langford had found inflammatory effects of the titanium miniplates, reported that tissue trauma, haematoma, tissue healing and mechanical instability between the implant and the surrounding tissues other than biocompability of the materials might be partly responsible for the histological changes seen in the tissues [29]. In addition some authors

have shown the biocampability of titanium alloys [7, 29]. However in the present study, the titanium miniplate groups showed significantly higher mortality rates than the control group ($P < 0.05$). Systemic effects of the titanium miniplates should be considered also with the local inflamatory effecets. As a matter of fact Bessho et al. reported that titanium released from miniplates might enter the vascular system and be deposited in distant organs such as the lungs, spleen, kidneys and liver [10].

The embryotoxic effects of titanium miniplates have not been investigated or documented in any academic report to date. The CHEST method is straightforward, replicable, and cost effective. This test could also be developed and combined with micronucleus assessments to determine DNA damage and genotoxic outcomes [23, 24].

On the other hand, the present study had some limitations. How could embrotoxic results of titanium plates effect the human biology was not suggested in this study. The CHEST model might not be analogous to the real clinical situation.

Conclusion

Miniplate removal is a controversial subject in maxillofacial surgery. Removal of miniplates has been discussed in terms of infection, discomfort, and wound healing in the literature [29]. A secondary surgery is needed to remove miniplates and this mandatory procedure often results in an uncomfortable postoperative period for patients.

Additionally, some quantity of metal ions from the implanted materials can be released via soft tissues.[29, 30, 31] It is worth noting that, despite the fact that some metals have toxic effects, this toxicity is not life-threatening. However more clinical and experimental studies have to be performed to evaluate the removal of the titanium miniplates.

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Psychosocial adjustment to lower-limb amputation: A review article

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Abstract

Introduction: Amputation is the loss of a part of an organ or all parts of an organ which is removed through surgery or is occurred due to trauma. The causes of amputation include trauma, infection, diabetes, vascular disease, cancer and other diseases. The impact of amputation on psychological situation and social and family relationships is undeniable, because physical disability also affects one's social and mental health, in addition to his/her psychological adaptation and compared to the ordinary people, these people are suffering more from social isolation. Therefore, any limb amputation not only is considered as a physical injury but is also followed by psychological- emotional damages.

Methodology: To prepare this paper we searched keywords such as adjustment, Lower-limb amputation and Psychological in the data banks of Scholar.google, Sciencedirect, Pubmed, Google, IranMedex, IranDoc and some Persian articles.

Conclusion: Following the amputation, patient experiences a wide range of conditions such as depression, anxiety, fatigue, long-term changes in recreational activities, economic burdens, medical costs as well as reactions of friends and family members, in addition to a wide range of emotional reactions. This situation, in the absence of adequate support from family and society, could result in non-adaptive responses of the patient. On the other hand, today the new methods of rehabilitation have turned the problem of disability from a personal tragedy to a social problem.

In this attitude, disability is a limitation imposed by the society which prevents these people from participation in social life. Although most researches have not found any relationship between individual –social characteristics and adaptation with the loss of limb, but studies show that men and the elderly could better adaptationcope with amputation compared to the young people and women, and the level of factors such as depression and anxiety is high for two years after the amputation, but these levels gradually decrease and reach to the norm of the general population. On the other hand, most studies have been conducted as quantitative and cross –sectional researches. In addition, some aspects of adaptationcoping with amputation have been neglected in researches untill now. The researcher believes that the main needs and concerns of these individuals and adaptationthe way they adapt with various problems are very vague and unknown for professional staff.

As qualitative researches can play an effective role in clarifying ambiguous and unknown areas and these types of researches have special effectiveness in answering to the questions containing human mentalities and interpretations and they are considered as the best ways to describe life experiences and relevant essential social processes, it is recommended to conduct qualitative researches in this field.

Key word: Psychosocial, adjustment, amputation.

Introduction

Socio-demographic Factors

Sex

Gender (sex) is also one of the psychological –social factors that can affect the outcome of amputation. In most conducted studies, no differences were found between mental health of men and women after amputation (1-6). But some studies have shown that women experience depression

more than men and they have poor emotional adaptation (7-10). Effect of Gender is not conclusive in the anxiety associated with the body image, although only one study points out that following amputation, women are more prone to the body image concerns than men (11).

Age

One of the potential criteria in adaptation with the amputation is the age. Various studies show that the rate of mental adaptation with amputation among young people is not desirable compared with adults (12-14), but the findings of some studies have shown that there is no statistically significant relationship between depression and age (4, 6, 15). In his study, Desmond did not find any significant relationship between age and depression, but the level of depression is reduced over time (12,16, 17).

Marital status

One of the important criteria in adaptation with amputation is marital status. Various studies show that the level of depression differs significantly in married and unmarried groups (4, 5, 15). This means that marital status is effective on depression and married people are less depressed than single ones. In explaining the impact of marital status on depression, it could also be said that the social relationships are more limited in people suffered from amputation compared with others, so marriage plays an important role in their mental health. In his study, Williams doesn't consider the marital status as one of the factors affecting the level of depression in people suffered from amputation. This finding can be attributed to the type of study (cohort) and population under study (6).

Social support

One of the factors affecting the adaptation with the lower- limb amputation is social support. Khademi showed that people with high social support had lower levels of depression and anxiety (4). According to the study conducted by Engstorm, family support can have a positive impact on adaptation with the amputation (18). Ziad's study showed that unmarried (single) patients and patients who had no social support have high levels of anxiety and depression (10). Various studies showed that symptoms of depression in people suffered from amputation increase with increased social isolation and decreased social support (3,17,19,20). Social support also had a direct impact on general adaptation with amputation among adolescents and young people. (21).

In a qualitative study, participants stated that one of the effective factors on promotion of successful rehabilitation is family support (22). Many quantitative studies point out that increase of social isolation and receiving less social support is associated with the reduction in quality of life and increased depressive symptoms (3, 19, 20, 23). General mechanism of literature in which social support (that increases mental health) is discussed, is build around two theoretical axes: buffer effect model and the direct effect model. Buffer effect model claims that the social support acts through the relationship between stressful life events and psychological distress and the direct effect model claims that the psychological health works with stress-free process (24).

Coping

Researches especially emphasize on the role of adaptation strategies in order to cope with the situation after amputation. Review of the literature indicates that the active strategies such as problem solving will result in positive psychological adaptation in most of the morbidities (14, 25). For example, Livneh points out that the increased problem-solving activity is negatively associated with depression and internalized anger and positively associated with the adaptation or coping with and acceptance of disability (14). However, the literature related to the adaptation and psychological coping with amputation, is a unique situation and the relatively limited researches have been done with different methodologies. At the first, studies in this field were logically conducted with small samples (14, 26, 27).

But later on, many researches were conducted exclusively on the coping with phantom limb pain (28-31). About the psychological –social adaptation with the amputation, Livneh believes that there are many factors effective on psychological- social adaptation or coping with the disability in chronic diseases (14) and coping strategies or ways used by people to manage experiences together with illness or injury play an important mediating role in

the psychological-social adaptation (coping) with amputation (32) and a person's ability to use these strategies and to enhance them is a key principle to prevent adaptation problems (16)

Amputation makes people to face with severe physical and mental-social challenges such as impaired physical function, use of the prosthesis and its accompanying problems, pain, change in employment and job status and changes in body image and self-confidence. Such stressors make it difficult for individuals to have a sense of emotional well-being and can lead to the reactions of non-adaptation or weak psychological-social adaptation (33, 34). Understanding these experiences and to feel empathic with the patient via understanding these experiences by the medical team can be useful in better care and helping patients to cope with this problem.

Reaction and responses to the amputation

Immediate reaction to the amputation varies and it depends on factors such as whether amputation was already planned or it was necessary following a chronic disease, infection or sudden trauma. Cause of amputation affects the mental condition of patient during the rehabilitation phase. He/she may experience the classic stages of grief when there is the time to think about the imminent loss of limb (35, 36). These stages include: denial stage (he/she often evidently refuses to participate in discussion and to answer fundamental questions about the program), anger stage (he/she may be angry with the medical team and say that he/she has fooled in announcing his/her agreement with the amputation, bargaining (he/she prevents surgery or delays it with thousands of reasons, including "I'm too tired," "I do not want major surgery." , depression (adopting a helpless, passive sense and drowning gesture) (37), and acceptance (he may not reach the rehabilitation process as long as he/she is ill (37). After having learned that amputation may be necessary, anxiety is often replaced with depression. This may be generalized anxiety (e.g., overt anger, decreased ability to sleep, silent rumination and social exclusion) or may lead to sleep disturbances and irritability. Not surprisingly, anxiety may be about the organ or limb which is going to be removed (38).

Phantom limb pain that may be familiar to many patients (those who have been called amputated individuals) is also expected. There may be hypersensitivity to the other people's negative attitudes to disability. This may be determined by the people suffered from amputation through the rejection of help and being indifferent to the questions concerning the expected performance level (38). Having contact with one of the religious figures can make it easier for them to accept the amputation (for example, a hospital chaplain). Other cases include perception (a positive aspect of life after surgery), self-hypnosis, exercising, pain relief after surgery and sense of more independence which facilitate the adaptation or adaptation with the inability period (39).

Patients who have amputation following an accident and the threat of infection may never spend these steps or they may only experience a very small part of these steps. Also they may not experience fear of anesthesia, surgery and waking during a few hours in a state of semi-consciousness. PTSD and depression screening after surgery and during the primary cares should be fully performed (40).

Conclusion

Returning back to the life after amputation is associated with many problems. These people often faces with psychological –social difficulties such as depression, feelings of hopelessness, low self-esteem, fatigue, anxiety, frustration, guilt, fear of the future status of the family and sometimes suicide due to the lack of adaptation with the new requirements. An individual suffered from amputation usually encounters with economic, social, personal, familial and environmental problems that make life more difficult for him/her. Unemployment incidence after amputation and direct and indirect health and medical costs resulted from amputation is higher in survivors of accidents and war and returning to the work is one of the major challenges of this group.

Many of these people have to change type of their jobs or to reduce their working time after injury. Following amputation, patients experience conditions such as prolonged fatigue, changes in recreational activities, economic burdens, and medical costs and also the reaction of friends and

family in addition to a wide range of emotional reactions which can be lead to non-adaptive reactions if they don't receive enough support from family and society. Thus, amputation is one of the major and traumatic incidents in the life of every individual which requires cooperation and assistance of a large number of people such as the surgical team, nursing, rehabilitation and finally family of these people to enable them to adapt with their new conditions (without feet) and have an independent life. Most of the researches conducted on amputation are quantitative studies which show that the level of factors such as depression and anxiety is high during the 2 years after amputation, but it then gradually decreases and reaches to the general population norm. But as the quantitative approach is incapable of measuring some phenomena and consequently by which it is not possible to describe aspects such as human values, culture, human relationships and communication, a new worldview is suggested as a supplement in the field of doing research which is called "The qualitative approach". This new worldview studies human phenomena rooted in the context of social science.

In this regard, Strobert and Carpenter (41) believe that it is not possible to summarize human phenomena in the form of mathematical formulas. Therefore, to achieve deep inner reality of human beings, more appropriate research strategies are required. Qualitative research can have an effective role in clarifying the ambiguous and unknown fields. Qualitative researches have specific effectiveness in answering to the questions that contain interpretations and mentalities of human and they are the best ways to describe life experiences and its major social processes. So, the use of a qualitative research for in-depth and comprehensive review of amputation seems necessary.

Following amputation, the patient experiences long-term conditions such as fatigue, changes in recreational activities, economic burdens, medical costs and reactions of friends and family, in addition to a wide range of emotional reactions which can lead to non-adaptive reactions if they don't receive enough support from the family and society. On the other hand, new methods of rehabilitation have turned the problem of disability from a personal tragedy to a social problem. In this trend, disability is a limitation from society which prevents the participation of these people in the social life. Although most researches did not find any relationship between individual characteristics and the adaptation with the amputation, but some studies show that old men (males) are adapted with the amputation better than young men and women. On the other hand, most of the studies have been conducted as quantitative and cross-sectional researches.

In addition, some areas of adaptation with the amputation have been so far neglected in the researches. The researcher believes that the major needs and concerns of these individuals and their adaptation with the various problems are very vague and unknown for the professional staff. Since the qualitative researches can play an effective role in clarifying the areas of ambiguity and unknown fields and these type of researches are effective in answering to the questions that contain human interpretations and mentalities and as they are considered as the best way to describe life experiences and its existing social processes, thus doing qualitative researches is recommended.

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Relationship between Premenstrual syndrome and depressive symptoms among nursing students

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Abstract

Aim: To determinate of relationship between Premenstrual Syndrome (PMS) and depression.

Material and methods: This cross sectional study was realized between from March-June 2011 among students of Nursing Department of Eskisehir Osmangazi University Health High School. The study group included 261 students. Premenstrual Assessment Form (PAF) was used for determination of PMS. PMS was diagnosed according to 1.7 and over PAF points. Depression was determined by Beck Depression Inventory (BDI). Depression doubtful case was approved that 17 and over BDI points. The chi square test and Spearman correlation were used in data analyses.

Results: The mean age was 20.46±1.51 years (range: 18-25). PMS and depression were found in 51 (%19.5%) and 49 (18.8%) students, respectively. The frequency of PMS was higher in smoking, depression doubtful, A type character, large family, and low educational level for father (for each one, $p<0.05$). Obtained points from between PAF and BDI were found significantly positive correlation ($r_s=0.280$; $p<0.001$).

Conclusion: PMS and depression were important health problems in this study group. The positive relation between PMS and depression was found. PMS and depression doubtful cases directed to secondary center for definitive diagnosis and treatment done would be helpful.

Key words: Premenstrual syndrome, depression.

Introduction

International Classification Diseases-10 is contained in PMS, and it defined as a cluster of mood, behavioral, and physical symptoms that occur during late luteal phase of the menstrual cycle and are relieved after the onset of menstruation (1, 2). In patients with PMS, the some signs and symptoms of depression include loss of interest in activities that were once interesting or enjoyable, loss of appetite, loss of emotional expression; a persistently sad, anxious or empty mood; feelings of hopelessness, pessimism etc. (3, 4, 5). The exact etiology of PMS is still unknown. Genetic, neurobiological factors and fluctuations in the levels of estrogen-progesterone are responsible for symptoms of PMS (6, 7, 8).

PMS leads to capacity loss of the individual, economic losses, increase in accident potential, and health problems such as anxiety, depression and therefore decreases the life quality (9, 10, 11, 12, 13, 14).

The prevalence of PMS in adolescent girls in the United States of America is 70–90% (15). In Turkey, the prevalence of PMS was found between 17.2% and 67.5% in the women in the age group of 15–25 (16).

In previous studies (17, 18, 19), the relationship between PMS and depression are mentioned.

The purpose of this research is to determinate of relationship between PMS and depression.

Material and methods

This cross sectional study was realized between from March-June 2011 among students of Nursing Department of Eskişehir Osmangazi University Health High School.

In three departments of this school; Nursing (387 students), Midwifery (193 students) and Health Care Management (50 students) (20). The study group was occurred with 261 (67.4%) students of nursing department. The other students (n: 126) were absent or rejected study in study period.

A questionnaire form was prepared according the literature (13, 16, 21, 22). This form was inclu-

ding some socio-demographic and menstrual characteristics.

The necessary permission for the study of school administration was received. Appropriate day and hour, the students gathered in classrooms. The students were informed about the study and verbal consent was taken from them. The questionnaire forms were filled by the students themselves. This process is continued for 30-35 minutes approximately.

PAF that developed by Halbreich et al. (23), was used for determination of PMS. Reliability and validity of the PAF were made by Dereboy et al. (24) in Turkey. The PAF consists of 95 items, each rated on a six-point scale of severity that focuses on the degree of change from the usual state that occurs premenstrual and disappears or returns to a usual level of severity during the full flow of menses. The focus is on change from usual state to discriminant premenstrual symptoms and impairment from chronic complaints (25). The interpretation of PAF; total score was divided into the number of questions. The obtained value is 1.7 and over was considered as PMS. For PAF questions, students were asked to take note of the last three pre-menstrual periods.

Determination of depression was made by BDI. The BDI was developed by Beck et al. (26) in 1961 and later modified by Hisli (27) in 1999 to suit the Turkish culture and norms. This scale includes four optional 21 questions. The answer for each item was evaluated as 0, 1, 2, and 3 points. The lowest number of points was accepted as '0' and the highest '63', with a cut-off point of 19. For BDI questions, students were asked to take note of the last two weeks.

Personality type was determined as Type A and Type B personality according to their testimony. Type A personality is usually competitive and has a high challenging spirit. They are always running and can hardly relax. Type B personality is almost the opposite of Type A (28).

Family income status was determined as good, middle, bad according to students' testimony. Students who smoked at least one cigarette per day were defined as smokers, whereas nonsmokers were defined as men who had never smoked or who had not smoked in the past 6 months (29), those consuming at least 30 grams ethyl alcohol in a week as alcohol consumer (30). Students were also examined for the existence of acne through physical inspection.

PMS history in family was accepted as PMS positive in their mothers or sisters.

If an adolescent had pain in the abdominal, groin, and lumbar region on the day before the menstrual period and/or the first day of menstrual period, it was considered to be dysmenorrhea (31). Regular menstrual cycle was evaluated as with equal intervals of menstrual cycle.

A woman who experiences variations of less than eight days between her longest cycles and shortest cycles is considered to have regular menstrual cycles. If an adolescent experienced menstrual bleeding in equal intervals between 21 and 35 days, it was evaluated as regular menstruation (normal); if the menstruation interval was less than 21 days, it was considered to be short; if the menstruation interval was more than 35 days, it was considered to be long. Menstruation of less than 2 days was accepted as short, between 2 and 6 days as normal, and more than 6 days as long (32, 33, 34).

Body mass index (BMI) was calculated by measuring their heights and weights. Those whose BMIs were 25 kg/m^2 and over were evaluated as overweight or obese (35).

SPSS 15.0 was used for statistical analyses. The statistical analysis was carried out using chi-square, and spearman correlation analysis. A value of $p < 0.05$ was considered statistically significant.

Results

The mean mean was 20.46±1.51 years (range: 18-25). The PMS frequency was found 19.5% (n: 51).

Socio-demographic parameters of students with/without PMS were presented in Table 1.

The smoking and alcohol habits were 29 (11.1%) and 10 (3.8%), respectively. The average BMI was 21.37±2.82 (min: 15, max: 33) kg/m^2. Depression frequency was found 49 (18.8%).

Habits and medical characteristics of students with/without PMS were presented in Table 2.

The average of the first menstrual age was 13.29±1.20 (min: 10, max: 18) years. The number of menstruation irregulation was 62 (23.8%). Dysmenorrhea frequency was 69.3% (n: 181).

Table 1. Socio-demographic parameters of students with/without PMS

Sociodemographic parameters	PMS			Statistical analysis X^2; p
	No (%)[a]	Yes (%)[a]	Total (%)[b]	
Age group (year)				
≤19	66 (79.5)	17 (20.5)	83 (31.8)	1.441; 0.486
20-21	92 (83.6)	18 (16.4)	110 (42.1)	
³22	52 (76.5)	16 (23.5)	68 (26.1)	
Living area				
Student hostel	83 (81.4)	19 (18.6)	102 (39.1)	3.348; 0.341
Home, single	37 (88.1)	5 (11.9)	42 (16.1)	
Home, with her family	45 (73.8)	16 (26.2)	61 (23.4)	
Home, with her friends	45 (80.4)	11 (19.6)	56 (21.4)	
Family type				
Nuclear	205 (82.3)	44 (17.7)	249 (95.4)	**Fisher; 0.003**
Large	5 (41.7)	7 (58.3)	12 (4.6)	
Social insurance				
No	13 (65.0)	7 (35.0)	20 (7.7)	Fisher; 0.081
Yes	197 (81.7)	44 (18.3)	241 (92.3)	
Family income status				
Bad	8 (57.1)	6 (42.9)	14 (5.4)	5.123; 0.077
Middle	136 (81.9)	30 (18.1)	166 (63.6)	
Good	66 (81.5)	15 (18.5)	81 (31.0)	
Mother's educational level				
Primary school or lower	133 (77.8)	38 (22.2)	171 (65.5)	1.801; 0.180
Secondary school or over	77 (85.6)	13 (14.4)	90 (34.5)	
Father's educational level				
Primary school or lower	71 (72.4)	27 (27.6)	98 (37.5)	**5.615; 0.018**
Secondary school or over	139 (85.3)	24 (14.7)	163 (62.5)	
Mother working status				
No	180 (82.2)	39 (17.8)	219 (83.9)	1.957; 0.162
Yes	30 (71.4)	12 (28.6)	42 (16.1)	
Pather working status				
No	53 (73.6)	19 (26.4)	72 (27.6)	2.395; 0.122
Yes	157 (83.1)	32 (16.9)	189 (72.4)	
Total	**210 (80.5)**	**51 (19.5)**	**261 (100.0)**	

[a]Percent for the row. [b]Percent for the column.

The average menstrual cycle duration 27.90± 7.21 (min: 15, max: 90) days. The average menstrual bleeding duration was 5.47±1.36 (min: 3, max: 10) days. Some characteristics related with menstruation of students with/without PMS were presented in Table 3. The average PAF point was 110.17±61.59 (min: 15, max: 338). The average BDI point was 10.45±8.84 (min: 0, max: 59). In our study, it was found significantly positive relation between PAF and BDI points (r_s=0.280; p<0.001). The correlation between PAF and BDI points were presented in Figure 1.

Discussion

PMS symptoms can begin at any age after menarche. Although the average age of onset has been reported as 26, in fact, PMS is a disorder that begins during youth. But young people are not severe enough to require treatment of symptoms (36). In our study, PMS was found 1 in 5 students. From Turkey, there are other studies (36, 37) reporting a high frequency of PMS among adolescent girls. It was found no relationship between age group and PMS (p>0.05). A cause of this result may be range

Table 2. Habits and medical characteristics of students with/without PMS

Habits and medical characteristics	PMS			Statistical analysis, X^2; *p*-value
	No (%)[a]	Yes (%)[a]	Total (%)[b]	
Smoking				
No	192 (82.8)	40 (17.2)	232 (88.9)	**5.764; 0.016**
Yes	18 (62.1)	11 (37.9)	29 (11.1)	
Alcohol consumption				
No	202 (80.5)	49 (19.5)	251 (96.2)	Fisher; 1.000
Yes	8 (80.0)	2 (20.0)	10 (3.8)	
Acne vulgaris				
No	99 (80.5)	24 (19.5)	123 (47.1)	0.000; 1.000
Yes	111 (80.4)	27 (19.6)	138 (52.9)	
Adverse life event history				
No	179 (81.0)	42 (19.0)	221 (84.7)	0.088; 0.767
Yes	31 (77.5)	9 (22.5)	40 (15.3)	
Overweight/obese				
No	188 (80.7)	45 (19.3)	233 (89.3)	0.000; 0.988
Yes	22 (78.6)	6 (21.4)	28 (10.7)	
Depression doubtful				
No	180 (84.9)	32 (15.1)	212 (81.2)	**12.730; 0.000**
Yes	30 (61.2)	19 (38.8)	49 (18.8)	
Type of personality				
A	100 (73.0)	37 (27.0)	137 (52.5)	**9.251; 0.002**
B	110 (88.7)	14 (11.3)	124 (47.5)	
Siling number				
0	3 (60.0)	2 (40.0)	5 (1.9)	3.512; 0.476
1	63 (81.8)	14 (18.2)	77 (29.5)	
2	64 (85.3)	11 (14.7)	75 (28.7)	
3	53 (77.9)	15 (22.1)	68 (26.1)	
4 and over	27 (75.0)	9 (25.0)	36 (13.8)	
Sibling order				
1st	110 (81.5)	25 (18.5)	135 (51.7)	2.921; 0.232
2nd	60 (84.5)	11 (15.5)	71 (27.2)	
3rd and over	40 (72.7)	15 (27.3)	55 (21.1)	
Total	**210 (80.5)**	**51 (19.5)**	**261 (100.0)**	

[a]*Percent for the row,* [b]*Percent for the column.*

of the study group age smaller than the average at onset of PMS.

Low socioeconomic status (SES) may increase stress factors. Thus, it can effect on occurrence of PMS. Family structure, social insurance, family income, mother's/father's educational level, and mother's/father's working status are parameters for SES. Some studies (38, 39) reported that PMS symptoms lower frequently in women with higher education level and women with high income. In our study, PMS frequency was higher in who living in large family than nuclear family ($p<0.05$). Additional, low level for father's education was a factor on the PMS frequency ($p<0.05$). Low educational level of the father suggested that low family income.

Smoking can alter levels of estrogen, progesterone, testosterone, which can be linked to the development of PMS. Some studies (40, 41) have found that smokers have shorter and more irregular menstrual cycles than non-smokers. In our study, smoker students were higher frequency of PMS than others ($p<0.05$). Forrester-Knauss et al. (42) reported that women with moderate to severe alcohol consumption were less likely to report PMS.

Table 3. Some characteristics related with menstruation of students with/without PMS

Some characteristics	PMS			Test değeri X^2; p
	No (%)[a]	Yes (%)[a]	Toplam (%)[b]	
Age at menarche (year)				
≤12	46 (78.0)	13 (22.0)	59 (22.6)	1.790; 0.409
13	81 (77.9)	23 (22.1)	104 (39.8)	
≥14	83 (84.7)	15 (15.3)	98 (37.6)	
Menstrual regularity				
Regular	163 (81.9)	36 (18.1)	199 (76.2)	0.765; 0.382
Irregular	47 (75.8)	15 (24.2)	62 (23.8)	
Menstrual cycle duration (days)				
≤20 gün	27 (79.4)	7 (20.6)	34 (13.0)	0.034; 0.983
21-34 gün	163 (80.7)	39 (19.3)	202 (77.4)	
≥35 gün	20 (80.0)	5 (20.0)	25 (9.6)	
Menstrual bleeding duration (days)				
≤6	165 (81.7)	37 (18.3)	202 (77.4)	0.541; 0.462
≥7	45 (76.3)	14 (23.7)	59 (22.6)	
Menstrual regulator drug using				
Yes	15 (75.0)	5 (25.0)	20 (7.7)	Fisher; 0.557
No	195 (80.9)	46 (19.1)	241 (92.3)	
Dysmenorrhea				
Yes	141 (77.9)	40 (22.1)	181 (69.3)	1.958; 0.162
No	69 (86.2)	11 (13.8)	80 (30.7)	
Premenstrual syndrome history in family				
Yes	46 (75.4)	15 (24.6)	61 (23.4)	0.906; 0.341
No	164 (82.0)	36 (18.0)	200 (76.6)	
Total	**210 (80.5)**	**51 (19.5)**	**261 (100.0)**	

[a]*Percent for the row,* [b]*Percent for the column.*

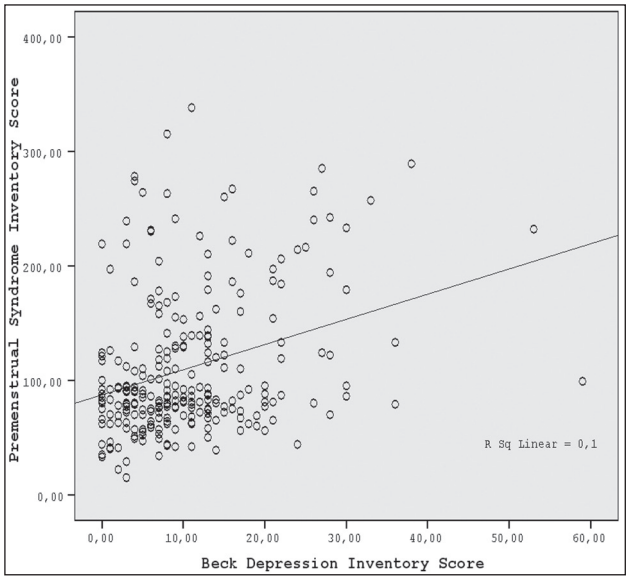

Figure 1. The correlation between PAF and BDI points

Any relation between PMS and alcohol consumption was not found in this study (p>0.05). This may be a reason that dose-response relationship for alcohol consumption ascertained for this study.

The adolescent with acne is higher frequency psychological signs. Wu et al. (43) reported that increase of prevalence of anxiety in patients with acne and positive relationship between severity of anxiety and severity of acne are reported. Therefore, thought it may be a relationship between acne and PMS, indirectly. But it was not found any relation between acne and PMS (p>0.05).

Masho et al. (44) reported that obese women had nearly a three-fold increased risk for PMS than non-obese women. But this study was not found any relation between obesity and PMS (p>0.05). Because this study has a young population, and there are very few obese.

Premenstrual symptomatology has earlier been linked to stress and a state-like alteration in the

perception of life events in the late-luteal phase of the menstrual cycle. Gonda et al. (45) reported that a significant positive association with the ratio of negative subjective life events. In our study, it was not found any relation between adverse life event history and PMS.

PMS frequency was higher in A type personality than others ($p<0.05$). This result may accept evidence to relation between stress factors and PMS. Güneş et al. (50) reported same result to us.

In our study, PMS couldn't find any relation with anyone character related menstruation (the first menses age, menstrual regulation, menstrual cycle duration, menstrual bleeding duration, menstrual regulator drug, dysmenorrhea, and premenstrual syndrome in family), (for each one; $p>0.05$). Conversely, Güneş et al. (46), Khella (47), and Demir et al. (48) reported association between PMS and menstrual irregulation. And PMS and dysmenorrhea were seen together in many women (49). Tomruk (50) reported positive relationship between PMS and dismenorrhea. Our study results can be explained as follows that this study tried to be a young group.

Although some women manifest premenstrual changes in mood and behavior, the changes are usually less severe. It is known that there is a relationship between PMS and psychiatric disorders especially affective disorders. 57-100 % prevalence of life time major depressive disorders in female patients with PMS has been determined. But premenstrual dysphoric changes have been reported in more than 2/3 of women with life time major depressive disorder (51). There is limited research on the relationship between major depression and premenstrual symptoms from studies with large sample sizes or from population-based studies (42). The temporal relationship between PMS and major depression has been investigated in several studies yielding conflicting results. Some studies have shown that women with PMS or Premenstrual dysphoric disorder (PMDD) have a higher percentage of past major depression than women without PMS or PMDD (52, 53, 54), while Hurt et al. (55) have reported contradictory results. There is also some evidence that women with PMDD might be at a higher risk to develop major depression in the future than women without PMDD (56, 57). In this study group, PMS frequency was higher in students with depression than students without depression ($p<0.05$). And additional, as expected, we found a positive correlation between PAF scores and BDI scores ($p<0.001$).

Limitations of this study are as follows: It's a cross-sectional. Therefore, the study might not show cause-effect relationships. The target group is limited to college students. For this reason, some features of PMS may have not appeared yet.

Conclusion

PMS and depression were important health problems in this study group. The positive relation between PMS and depression was found. PMS and depression doubtful cases directed to secondary center for definitive diagnosis and treatment done would be helpful.

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A spilt dose of levonorgestrel versus single dose of levonorgestrel for emergency contraception: A randomized controlled trials

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Abstract

Introduction: Unwanted pregnancy is a global concern. Morning-after pill is an important method to prevent unwanted pregnancy. This study was designed to compare effectiveness and safety of single dose of levonorgestrel versus spilt doses in health centers of Yazd, Iran.

Materials and methods: Through conducting a Randomized Controlled Trial in health centers of Yazd, during 2009-2010; one hundred and ten women who seeking emergency contraception following a single act of unprotected intercourse were randomly assigned to receive two regimes: a single dose 1·5 mg levonorgestrel (n=53) orally or in spilt doses 12 h apart levonorgestrel (0.75 mg each time, n=57). Data were gathered by questionnaire at the beginning of the study, three weeks and three month later. Percentage of success rate and side effects were recorded and analyzed.

Results: The failure rate of two regimnes was determined 4% in single dose and 7 % in split dose (P=0.375). Side effects were reported in 33% in single dose group and 46% in split dose (P=0.17). There was no significant statistical difference between time of drug administration in menstrual cycle and menstrual pattern (P=0.192).

Conclusion: Single dose of levonorgestrel was more effective than two doses. Both regimens had minimal side effects.

Key word: Levonorgestrel, emergency contraception, unprotected intercourse.

Introduction

Morning-after pill refers to the use of drugs as an emergency contraception to prevent unwanted pregnancy (1, 2). World Health Organization (WHO) has reported, 50 million pregnancies are discontinued every year in the world (3). Emergency contraception prevents the establishment of pregnancy after unprotected intercourse (4, 5). A trial reported by WHO (1998) introduced "levonorgestrel only" as the gold standard for morning after pill (6).

The mechanism of action of morning after pill is doubtful and may vary depending upon the day of the menstruation cycle, in which the drug is administered (7-9). There are many controversial opinions concerning the probable mechanisms of emergency contraception which prevents unwanted pregnancy. Lately, the International Federation of Gynecology and Obstetrics and the International Consortium for Emergency Contraception released a Joint Statement declaring that "Inhibition or delay of ovulation should be their primary and possibly only mechanism of action" (10).

The typical regimen, 1.5 mg of levonorgestrel either as a single dose or in two doses 12 h apart, can be administered within 120 h of an unprotected intercourse as emergency contraception (11, 12). It was ordered over 10 years ago that a high dose of levonorgestrel alone, 0.75 mg taken twice at 12 h intervals (13), was equal or more effective than the Yuzpe regimen and was associated with less side effects (14).

The rate of efficacy and side effects of two regimen of emergency contraceptive pill including single dose of levonorgestrel versus spilt doses has been compared in several studies. Many investigators found that two regimen of Levonorgestrel (single dose and split dose) were similar in effectiveness (15, 16), Recently FDA recommended single dose of levonorgestrel (17).

Several studies were conducted regarding unwanted pregnancy and emergency contraception in Iran. According to Erfani and McQuillan (2008), unwanted pregnancy rate is 34% in Iran. Also Fa-

rajkhoda et al. (2009) reported a success rate 100% of single dose of levonorgestrel versus Yuzpe regimen (18). Considering reported Total Fertility Rate (TFR) two children for every woman and noticeable percentage of unwanted pregnancy in Iran (19) and lack of enough studies in this regard in Iran, this study was designed to compare the effectiveness and safety of two regimen of emergency contraceptive pill (single dose of levonorgestrel versus spilt doses) after unprotected intercourse to prevent unwanted pregnancy in order to introducing effective and safe emergency contraception method in Iran.

Methods

The study's protocol was approved by the committee of Shahid Sadougi University of Medical Sciences, Yazd, Iran. This randomized controlled trial study was registered in http: //www.irct.ir, IRCT with registration code: IRCT201106186826N1. Women who were attended in family planning unit of health care centers of Yazd from to seeking for emergency contraception were selected for participating in the study. Informed consent was obtained from all women who participated in the study. Healthy women with normal menstrual cycles, who had only one unprotected sexual intercourse within five days ago and they had used condom for protection against pregnancy after previous unprotected intercourse, no current use of hormonal contraception and age between 16-45 were considered qualified for enrolment in this randomized, multicentre trial study according to the defined inclusion criteria of the study. Acting of intercourse after 120 hours without protection against pregnancy (with condom usage) and other self administrated additional treatment for emergency contraception were determined as exclusion criteria.

One hundred and ten women were randomly (by computer-generated random table) assigned to receive two regimens: a single dose of 1·5 mg levonorgestrel (n=53) orally or in spilt doses 12 h apart levonorgestrel (0.75 mg, n=57) (tab longel 0.75 gm: Iran hormone company).

After delivering levonorgestrel pill to women, a diary was given to women for recording the side effects such as nausea, vomiting, vertigo, headache, breast tenderness and gastric pain in the first visit. Midwives in family planning unit of health care centers were recorded needed data by phone three weeks later. Three evidences might be happened: (1) normal menstruation, (2) pregnancy and (3) delay menstruation. Women were advised not to have further acts of coitus before menstruation return.

Follow-up visit was arranged three weeks and three month after intervention. βhCG test were requested for women who had delayed in their menstruation, three weeks later intervention. Negative result of βhCG test was considered as treatment success rate. If the βhCG test was reported positive, another additional visit was done in three month later for diagnosis of Ectopic Pregnancy.

The data were analyzed by means of simple percentages for categorical variables, descriptive statistics for continuous variables, and chi-square or Pearson tests for qualitative variables at the 95% confidence level.

Results

The study main results were to determine pregnancy rate and side effects in the two regimes of Levonorgestrel. Two groups were similar in demographic characteristics. The mean age of women was 27.20±5.8 years in single dose and 26.45±6.0 in split dose of Levonorgestrel (P=0.079). The number of previous pregnancy (Gravida) was calculated 1.67±1.08 in single dose and 1.52±1.35 in split dose of Levonorgestrel (P=0.380). Duration of menstruation in each cycle was determined 6.35±1.44 days in single dose and 6.78±1.49 days in split dose of Levonorgestrel (P=0.176). Interval between coitus and drug administration was reported 17.08±2.3 h in single dose and 16.22±2.1 h in split dose of Levonorgestrel (P=0.962). There was not any significant statistical differences between two groups in mentioned variables (Table 1).

In our study recent contraception method showed 25 women (22.7%) in single dose and 43 women (39.1%) in split dose of Levonorgestrel used condom. There was significant statistical differences between two groups in type of contraception variables (P=0.002) (Table 2).

Menstrual bleeding after using emergency contraception was occurred 10.84±1.08 days after single dose and 9.93±1.35 days after split dose of Levonorgestrel in which there was not any significant statistical differences between two groups (P=0.962).

Table 1. Demographic characteristics of women in two regimnes of Levonorgestrel

Group Variable	Single dose	Spilt dose	P value
Age	27.20±5.81	26.45±6.03	0.079
Gravida	1.67±1.08	1.52±1.13	0.380
Duration of menstruation after intervention	6.35±1.44	6.78±1.49	0.176
Interval between coitus and drug taking	17.08±2.34	16.22±2.14	0.962

Table 2. Current method of contraception in two regimens of Levonorgestrel

Group Type of contraception	Single dose	Spilt dose	P value
Condom	25 (22.7%)	43 (39.1%)	0.002
Withdrawal	28 (25.5%)	14 (12.7%)	
Total	53 (48.2%)	57 (51.8%)	

Table 3. Outcome after drug administration in two regimens of Levonorgestrel

Group Outcome	Single dose	Spilt dose	Total	P value
Normal pregnancy	4 (7%)	2 (4%)	6 (5%)	0.375
Normal menstruation	45 (86%)	50 (88%)	95 (86%)	0.478
Irregular menstruation	4 (7%)	5 (8%)	9 (8%)	0.478
Total	53	57	110	

Table 4. Side effect after drug administration in two regimens of Levonorgestrel

Group Side effect	Single dose	Spilt dose	Total	P value
Nausea	2 (3.8%)	11 (19.3%)	13 (12%)	0.17
Nausea & Vomiting	3 (5.7%)	0 (0%)	3 (1.9%)	
Headache	4 (7.5%)	7 (12.3%)	11 (10%)	
Vertigo	1 (1.9%)	4 (7.0%)	5 (4%)	
Breast tenderness	1 (1.9%)	1 (1.9%)	2 (1%)	
Gastric pain	3 (5.7%)	1 (1.8%)	4 (3%)	
Others	3 (5.7%)	2 (0%)	3 (2%)	
Without symptom	36 (67.9%)	31 (54.4%)	67 (66%)	
Total	53 (100%)	57 (100%)	110 (100%)	

The main aim of the study was to determine the failure rate of two regimes of Levonorgestrel. Pregnancy was reported in 4% (two women) in single dose and 7 % (four women) in split dose of Levonorgestrel. There was not any significant statistical difference between two groups in this regard (P=0.375) (Table 3). In addition, Ectopic Pregnancy was not reported in pregnant women in two groups in three-month follow up visit.

Normal menstruation was occurred in 45 women (86%) of single dose and 50 women (88.0%) of Levonorgestrel in the next menstrual cycle in unpregnant women (P=0.478). Irregular menstruation was reported in four women (7%) in single dose and five women (8 %) in split dose of Levonorgestrel (P =0.478) (Table 3).

In 19 women (33%) in single dose and 32 women (46%) in split dose of Levonorgestrel minor side effects were reported. Nausea, vomiting, headache, breast tenderness, and gastric pain were reported as notable minor side effects There is not any significant difference between time of drug administration in women's menstrual cycle and their menstrual pattern (P=0.17) (Table 4).

Time from last menstruation (LMP) and drug administration was showed in Table 5. It confirmed that the most percent of pregnancies occurred in mid cycle (P=0.046) (Table 5).

Table 5. Interval betwwen last menstrual period and drug administration in two regimens of Levonorgestrel

Out come Cycle day	Normal pregnancy	Pvalue
1-10	1	0.046
11-19	4	
20-34	1	
Total	6	

Discussion

In this randomized controlled trial the effectiveness of the single dose of levonorgestrel versus spilt dose were compared. The main outcome was to determine the pregnancy rate in the two treatment regimes. In our study pregnancy rate was reported 4% in single dose and 7 % in split dose of Levonorgestrel. Failure rate in single dose of levonorgestrel group was reported 2·6% by Glasier et al. (2010) (1). This result was similar to our finding. Also Wai Ngai et al. (2005) reported that failure rate was 1.9% in the single dose of levonorgestrel group and 2.0% in the split dose of levonorgestrel group (P=NS) and they mentioned split dose of levonorgestrel regimen was as effective as the 12 h regimen for emergency contraception up to 120 h after unprotected intercourse (15). Arowojolu et al. (2002) stated that the single dose of levonorgestrel was more effective than the dose (16). Kook in his study showed that split dose of levonorgestrel was as effective as single for emergency post-coital contraception up to 120 h after unprotected intercourse (P=NS) (20). Von Hertzen et al. (2002) in a WHO multicentre randomized trial, showed that the pregnancy rate was 1.5% in single dose of levonorgestrel, and 1.8% in split dose of levonorgestrel (21). Our findings confirmed single dose of levonorgestrel was more effective than split dose of levonorgestrel, although statistical significant differences was not observed.

Determining the side effects of two regimens was the study's second aim. In our study, serious side effects were not observed and both regimens had minimal side effects.

The American College of Obstetricians & Gynecologists (ACOG) Practice Bulletin (2009) reported less efficacy and more side effects of Yuzpe regimen in compare with levonorgestrel alone, so Yuzpe regimen is no longer commonly used (22). Uncommon reported side effects are including nausea and vomiting, dizziness, fatigue, headache, breast tenderness, and gastric pain. These side effects may be treated symptomatically (16). Any death or serious complications were not associated with these drugs. Nausea and vomiting are two side effects of the oral combined hormonal drugs, and are seen more than the levonorgestrel regimen (nausea was 43% in Yuzpe regimen versus 18% in levonorgestrel regimen and vomiting was 16% in Yuzpe regimen versus 4% in levonorgestrel regimen) (23). levonorgestrel was associated with a lower rate of side effects than Yuzpe regimen (nausea: RR= 0.43, 95% CI 0.39-0.48 and vomiting: RR= 0.24, 95% CI 0.18-0.31) (3). Glasier et al. (2010) reported that side effects of ulipristal acetate and levonorgestrel were similar (1). Wai Ngai et al. (2005) recommended the second dose of levonorgestrel could be given in a flexible plan (12–24 h) to make it more comfortable for the women. He confirmed both regimens of levonorgestrel had minimal side effects. He suggest more study need to obtain which plan is more effective and have less side effects (15). Also Johansson et al. (2002) showed that breast tenderness and diarrhea after taking levonorgestrel occurred more frequently in the 12 h regimen comparing with the 24 h regimen. However, the incidence of these side effects was low and not clinically significant even in the 12 h regimen (24). The women, who took single dose of 1.5 mg levonorgestrel, experienced more headache, breast tenderness and menorrhagia than spilt dose of 0.75mg levonorgestrel, but he confirmed that both regimens of emergency contraception had minimal side effects (16). As our results showed we recommended single dose of levonorgestrel because both regimes had low side effect and forgetfulness is not seen in single dose.

In our study, interval between coitus and drug administration was not statistically significant in two groups and all of women took the regimens within 72 h following unprotected intercourse. If levonorgestrel regimen would be given earlier following unprotected intercourse, the much more efficacy would have observed (25).

Von Hertzen et al. (2001) reported that further acts of intercourse (with and without contraception) between treatment and expected menstruation resulted in higher pregnancy rates (21). In

contrast, Wai Ngai et al. (2005) study results showed that further acts of intercourse significantly increased the pregnancy rate in women who underwent the 12 h treatment, comparing those who underwent the 24 h treatment (15). In our study, having not further acts of intercourse was considered as one of the study's exclusion criteria.

Irregular bleeding is not unusual in the month after treatment, and has been reported in 16% of women in the first week after using emergency contraception pills (21, 26) ACOG Practice Bulletin (2010) and Devoto et al. (2005) observed significant but transient changes in menstrual cycle characteristics after emergency contraception including cycle length, duration of menses, menstrual appearance and incidence of inter menstrual bleeding. Cycle characteristics returned largely to baseline values in the next complete menstrual cycle (23, 27). We observed irregular menstruation in four women (7%) of single dose and five women (8%) of spilt dose.

There were few data on the risk of ectopic pregnancy subsequent of levonorgestrel treatment for emergency contraception. Wai Ngai el al. (2005) reported the occurrence of 40% in their study. None of these pregnancies was ectopic (15). Also, Sheffer-Mimouni el al. (2003) reported three cases of ectopic pregnancy after taking levonorgestrel as emergency contraception (28). Until that time, there was no evidence for association between levonorgestrel prescription and increasing risk of ectopic pregnancy (15). Wai Ngai el al. (2005) findings confirmed our study. In our study, women with positive pregnancy test were visited three month after taking levonorgestrel and no ectopic pregnancy was occured.

According to the study results single dose of levonorgestrel was more effective than split dose but both regimens had minimal side effects. Therefore, a single dose of levonorgestrel could be used as an effective and safe emergency contraception method with less probability of lethe and comforting in usage for women who seek emergency contraception pill.

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Fatigue and associated factors in patients on hemodialysis treatment in Turkey

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Abstract

Aim: The aim of this study was to identified level of fatigue in patients on hemodialysis and examined associations between fatigue and personal and illness-related characteristics.

Methods: A cross-sectional, descriptive and correlational design was used. The *Piper Fatigue Scale* (PFS) and demographic questionnaire was completed by 222 patients on hemodialysis at two dialysis units.

Results: The mean age was 53.41±14.37. Most of the patients were male (55.9 %) and married (75.2 %). Fifty percent of (n=110) the participants had an income equal to their expenses. Only seventeen (7.7%) were in employment during data collection. Most of patients (72.5 %) reported severe levels of fatigue for total of the PFS. No significant differences on fatigue scores were identified by gender, educational, economic status and chronic disease (p>0.05).There were significant differences between mean fatigue scores for marital, employment status and living with together people in home (p<0.05). There was a positive weak significant correlation between the mean fatigue scores and age (r=0.20, p=0.000). A statistically significant relationship were not found among fatigue score and length of time on hemodialysis (r=-0.012, p=0.861) and frequency of hemodialysis (r=-0.023, p=0.733).

Conclusion: Fatigue are highly severe in patients receiving HD. It can be reduced by assisting patients to develop coping strategies. Nurses should educate the patients about management of fatigue and practical strategies for managing fatigue patients on hemodialysis need to be developed, implemented and evaluated

Key words: Hemodialysis, fatigue, nursing, the Piper Fatigue Scale.

Introduction

In Turkey, hemodialysis is the most commonly used renal replacement therapy (RRT) method and 82.7% of the 14.590 individuals with end stage renal disease (ESRD) receive hemodialysis (1). Additionally the patients often suffer from complications such as cardiac disease, hypertension, anemia, and renal osteodystrophy. These complications may result in the emergence of physical and emotional symptoms. Symptoms commonly mentioned by dialysis patients are fatigue, pruritus, muscle cramp, and nausea (2,3). These symptoms may also negatively affect the quality of life patients receiving hemodialysis and, as such, the assessment and management of these symptoms are an important issue for nephrology nurses (2,4) Fatigue is a prevalent and severe symptom in patients on hemodialysis (4,5,6,7,8,9,10). Researches have shown that 70%- 85% of patients who receive dialysis treatment experience symptoms of fatigue (2,3,9,10). However, there is no accepted definition of the phenomenon of fatigue. One definition is that "it is whatever the patient says it is, whenever he says it is" (11). Ream and Richardson (1996) using concept analysis, defined fatigue as a "subjective and unpleasant symptom that incorporates total body feelings ranging from tiredness to exhaustion creating an unrelenting overall condition that interferes with the individual's ability to function to their normal capacity" p.527. (12).

Although not all of the factors that cause fatigue in patients on hemodialysis are known clearly, many factors are believed to play a part. The relationship between physiological factors (Hb, albumin, urea reduction ration (URR), the urea kinetic equation (Kt/V), duration of hemodialysis, length of time the patient has been on hemodialysis, frequency of HD per week), psychological factors (depression, anxi-

ety, sleep quality) and situational factors (age, gender, marital status, educational level, employment, economic status etc.) have been investigated in regard to fatigue in patients on hemodialysis. McCann and Boore (2000) and Bonner et al. (2008) found that there was no relationship between fatigue and gender (6,5). On the other hand, O'Sullivan and McCarthy (2007) and Liu (2006) reported that females were significantly more fatigue prone than males (7,4). In contrast; one study showed that men were significantly more fatigued than women (13). A relationship has not been identified between fatigue and age (5,7). However, some studies have reported that age was identified as a factor associated with fatigue in patients on hemodialysis (4,10). In studies that have examined the relationship between marital status and fatigue, no relationship was found (5,6,7). McCann and Boore (2000) found that fatigue was associated with physical function, sleep problems and depression (6). In some studies, no relationship was found between length of time on dialysis and the presence of fatigue (6,7). Some studies have found that employment status was not related to reported levels of fatigue (6,7). Obviously, fatigue is a prevalent symptom in patients on hemodialysis but making a clear statement about the factors responsible for fatigue is difficult.

Nurses are in a strategic position to provide patients' need for holistic care and are responsible for assessing the progress of patient with ESRD and the impact of the illness on the patient. Also, nephrology nurses are ideally placed to identify, assess, assist and teach strategies to patients in managing fatigue symptoms during the dialysis through education. Patients can begin to explore how to manage fatigue and whether these strategies are effective, thereby promoting patient empowerment (14).

The aim of the present study were to identify the following:

- Fatigue level in patients on hemodilaysis
- The socio-demographic and disease-associated factors that affect fatigue
- Correlations between fatigue and some characteristics (age, duration of disease, length of hemodialysis, and frequency of hemodialysis).

Materials and methods

Design and sample

This descriptive study was carried out between February 2007 and September 2008 in two dialysis unit in İzmir, Western Turkey.

A convenience sample of patients was obtained from all patients who were receiving hemodialysis in two dialysis units. The inclusion criteria were a) age 20 years or more, b) regular hemodialysis treatment for at least 6 months c) no systemic diseases causing or increasing levels of fatigue such as systemic lupus erythematosus or cancer, d) no auditory or visual impairment, and e) willingness to participate in the study.

Ethical consideration

Written approval was obtained from the local ethics committee of the nursing college and two dialysis units to conduct the research. All patients were informed by the researcher about the aims of the study, and verbal consent was obtained for participation.

Study instrument

The data were collected by demographic questionnaire and the Piper Fatigue Scale (PFS)(15). A demographic questionnaire form was also developed by the author to obtain data related to patients' socio-demographic (age, gender, marital status, educational level, perceived economic level, employment status, living with other people in their home) and illness-related variables (duration of illness, length of time on hemodialysis, frequency of hemodialysis).

Fatigue was measured by the revised PFS. The revised PFS was the first multidimensional subjective fatigue measure with reliability and validity established in patients with cancer. The revised PFS, used to measure perceptions of fatigue in patients in this study, consists of 22 numerically scaled, 0 to 10 items that measure 4 dimensions of subjective fatigue. The 4 subscales and the symptoms or problems that they assess are as follows: behavioral/severity subscale (6 items)-severity and degree of disruption to activities ofdaily living due to fatigue, affective subscale (5 items)-emotional meaning attributed to fatigue, sensory subscale (5 items)-physical symptoms of fatigue, and cognitive/mood subscale (6 items)-mental and

emotional symptoms of fatigue. These 22 items are used to calculate the 4 subscale/dimensional scores and total fatigue scores. To calculate subscale scores, individual items on each subscale were summed and divided by the number of items on the respective subscale. The total fatigue score was calculated by adding scores of the 22 items together and dividing the sum by 22. A PFS score of > 7 is indicative of severe fatigue. In this study, the fatigue level of most of the participants' was > 7. In the original study performed by Piper et al, the Cronbach alpha validity coefficients for the subgroups ranged from .92 to .96. The standardized alpha for the entire scale was .97. The validity and reliability of the tool for the Turkish population was conducted by Can et al. (2004) and the tool's Alpha value was determined to be 0.92 (16). In our study the Cronbach alpha validity coefficients for the subgroups of the PFS were between .82 and .84.

Data collection

The researcher collected the data by using a face-to-face interview technique to help increase the accuracy of collected information. The patients were interviewed alone. During data collection, the questions were read to the patients and were marked on the questionnaires.

Statistical analysis

Data analysis was performed using SPSS software (version 11.0 of the SPSS). Descriptive statistics, means, frequencies, and percentages were used to show the distribution of personal characteristics, illness-related characteristics, and scale scores. In comparing the mean values of the scales for selected personal (gender, marital status, educational level, perceived economic level, employment status, living with other people in their home) and illness-related variables (duration of illness, length of time on hemodialysis, frequency of hemodialysis), parametric test were utilized. Furthermore, the relationship between perceived fatigue and some characteristics (age, duration of illness, length of time on hemodialysis, frequency of hemodialysis) were examined using Pearson's correlations. For all statistical analyses the significant levels were set as $p<.05$.

Results

A total of 235 patients fullfilled the study inclusion citeria. Among them 13 patients declined the study. A total of 222 patients completed the study. A summary of the results for personal and illness-related characteristics is presented in Table 1, and for the level of fatigue in Table 2, comparisons of mean scores of fatigue according to these characteristics are shown in Table 3, and the relationship between fatigue and these variables is given in Table 4.

Table 1. Characteristics of the Sample

Characteristics	Mean (SD)	
Age/*years*		
Time since diagnosis (*months)*	53. 41 (14.37)	
Length of time on hemodialysis(-*months)*	74.59 (63.64)	
Frequency of hemodialysis(*days / week)*	48.28 (43.24) 2.99 (1-3)	
Gender	**n**	**%**
Male	124	55.9
Female	98	44.1
Age		
60 years and older	90	40.5
40-59 years	90	40.5
20 - 39 years	42	18.9
Marital status		
Married	167	75.2
Divorced/widowed	28	12.6
Single	27	12.2
Educational status		
Illiterate	28	12.6
Primary school	145	65.3
Secondary school	30	13.5
High school	3	1.4
University	16	7.2
Perceived income level		
Income equal to expenses	110	49.5
Income less than expenses	99	44.6
Income higher than expenses	13	5.9
Work status		
Unemployed	205	92.3
Employed	12	5.4
Employed part- time	5	2.3
Living with other people		
Spouse + children	99	44.6
Spouse	54	24.3
Children	26	14.4
Other	32	11.7
Single	11	5.0
Total	222	100

Table 2. Fatigue level of patients

Level of fatigue Score	n	%
0 (no fatigue)	-	-
1- 3 (mild fatigue)	36	16.3
4-6 (moderate fatigue)	25	11.3
> 7 (severe fatigue)	161	72.5

Table 3. Personal characteristics associated with fatigue of hemodialysis patient

Characteristics	Piper Fatigue Scale (PFS) Total PFS Mean (SD)	F	p
Marital status			
Married	8.15 ± 3.63	5.52	0.05*
Divorced/widowed	6.33 ± 4.57		
Single	9.63 ± 2.68		
Education level			
Illiterate	8.49 ± 2.90	0.33	0.85
Primary school	8.18 ± 3.75		
Secondary school	7.44 ± 4.16		
High school	8.62 ± 3.68		
University	8.04 ±4.24		
Economic status			
Income=expenditure	8.59 ±3.51	2.62	0.07
Income<expenditure	7.93 ± 3.85		
Income>expenditure	6.23 ± 3.72		
Employment status			
Unemployed(n= 205)	5.58 ±4.52	3.73	0.02*
Employed(n=12)	6.35 ±3.43		
Employed part-time (n=5)	8.32 ±3.63		
Living with other people			
Spouse +children	8.51 ±3.71	3.37	0.01*
Spouse	10.10 ± 2.04		
Children	7.66 ± 3.63		
Other	8.94 ± 3.34		
Single	6.97 ± 4.59		
Gender		**t**	**p**
Male	7.90 ± 3.63	t=1.104	p=.273
Female	8.45 ± 3.76		
Chronic disease			
No	8.37 ±3.87	t=-1.035	p= .308
Yes	7.86 ± 3.47		

** p < 0.05*

Table 4. Correlation Coefficients for Age, Duration of Illness, Length of Time on Hemodialysis and Frequency of Hemodialysis Associated with Fatigue

	Fatigue score *r*	*p value*
Age	0.24 **	.000
Duration of illness	- 1.38 *	.040
Length of time on hemodialysis	-0.012	.861
Frequency of hemodialysis	-0.023	.733

*** p < 0.01* ** p < 0.05*

Participants' characteristics

Table 1 lists the personal and illness-related characteristics of patients who completed the study (n=222). The sample consists of 124 (55.9%) male and 98 (44.1%) female patients. Ages ranged from 20 to 82 years with a mean of 53.41 (Standard deviation (SD)=14.37). Most subjects were married (75.2%), had been through primary school (65.3%). Fifty percent (n=110) of the participants had an income equal to their expenses, 44.6% (n=99) an income less than their expenses, and 5.9% (n=13) an income higher than their expenses. Only seventeen (7.7%) were in employment, twelve full-time and five part-time. Forty-five percent (n=99) of the patients were living with their spouse and children.

Fatigue level

As seen in Table 2, most of the patients (72.5%) reported severe levels of fatigue on the PFS. In this study, patients reported that they had experienced fatigue at a mean score of 8.14 (SD 3.70), indicating a generally high level. Patients who were found to have experienced fatigue were asked what causes of fatigue and which measures they used to cope with it. They said that the causes of fatigue included hemodialysis treatment (16.3%) and arrival and departure (7.2%) to dialysis units. Thirty percent stated that they were asleep for coping with it. In addition to sleeping, other measures used included resting and reading a book.

Fatigue and socio-demographic/ disease-associated factors

There were statistically significant differences in mean fatigue scores between groups, for marital status, employment status and living with other peoples. Compared to patients' marital status, the fatigue scores were higher in single patients than in married or divorced/widowed patients.

The fatigue scores were higher in employed part time patients than in unemployed patients (F=3.73; p=0.02). Statistically significant differences were found between scores on the PFS and those living other people. Patients living with their spouse had higher the fatigue scores than those living singly (F=3.73; p=0.01). There was no statistical difference in gender (t=1.104, p=.273), educational level (F=0.33; p=0.85), economic status (F=2.62; p=0.07) and chronic disease status (t=-1.035; p= .308) of patients on hemodialysis in relation to the fatigue scores.

Relationships between fatigue and some characteristics

Table 4 outlines the relationships between fatigue scores and personal and illness-related characteristics. The relationships between fatigue and selected variables (age, duration of illness, length and frequency of hemodialysis) was analyzed using a Pearson product-moment correlation coefficient. A positive weak relationship was found between fatigue and age of respondents and these results were statistically significant (r= 0.24, p=.000). There was also a statistically significant relationship between fatigue and duration of illness (r =-1.38, p=.040) indicating that as the duration of the illness increased, fatigue levels decreased.

No statistically significant relationship was found between fatigue score and length of time on hemodialysis (r=-0.012, p=.861). Also, there was no a statistically significant relationship between fatigue score and frequency of hemodialysis (r=0.023, p=.733)

Discussion

To our knowledge, this is the first study to measure fatigue in people receiving hemodialysis using the PFS.

Fatigue level

Fatigue was the most common symptom reported by patients on hemodialysis,and it can be measured using an instrument, such as the PFS. This study indicated that respondents were generally severely fatigued. This result is consistent with previous research, which found that most patients who receive maintenance hemodialysis experienced a moderate or severe level of fatigue (4,5,6,8,10). Comparably, Bruiner and Graydon (1993) found low levels of fatigue in in patients on hemodialysis measured by the fatigue subscale of the Profile of Mood States (13). It is clear that fatigue is a prevalent symptom among hemodialysis patients. Several factors have been proposed as associated with the fatigue experienced with ESRD. These include prescribed medications and their side effects, nutritional deficiencies, physio-

logical alterations, particularly abnormal urea and hemoglobin (Hb) levels, psychological factors such as depression, sleep dysfunction and factors associated with hemodialysis treatment (low dialysate sodium and excessive ultrafiltration). In our study, The factors cause the fatigue in patients on hemodialysis were hemodialysis treatment and arrival and departure to the dialysis unit.

We think that nurses and other health care team members need to be aware of these factors. To provide holistic care, nurses should screen for the presence and severity of fatigue at the initial contact with patients, at appropriate intervals thereafter, and as clinically indicated. If fatigue is reported during screening, they should be focus on the history and physical examination of the patients' disease, treatment status, current medication, review of systems and in-depth fatigue (onset, pattern, duration, change over time, associated factors or alleviating factors, interference with function). They should also assist patients to develop coping strategies to prevent fatigue such as exercise, energy conservation, activity management, optimizing sleep quality and using relaxation, yoga, acupuncture, or distraction methods. In this study, sleeping, resting and reading a book have been reported to be benefical in helping patients cope with fatigue.

Most of these strategies have been identified as effective in oncology patients including participating in exercise programs, managing activity, optimizing sleep quality and receiving education (17). We suggest that these strategies for future research should include longitudinal measurement of fatigue in both hemodialysis and peritoneal dialysis patients from the date of diagnosis until end of their lives.

Fatigue and socio-demographic/ disease-associated factors

In the present study married and divorced/widowed participants reported significantly higher fatigue than single subjects on the PFS. Varying results have been obtained from other studies regarding the association between marital status and fatigue (5,6). In the same studies no significant differences on fatigue scores were identified relating to marital status. Liu (2006) established that unemployed participants reported significantly higher fatigue than employed subjects (4). This result is consistent with the findings of the present study. However, the data of the present study are in contrast with other studies (6). The association between employment status and hemodialysis treatment is unclear in the present study. Patients who had higher fatigue scores might lack of energy to endure requirements to a job. Or, resting, sleeping at home may decrease the amount of physical activity and social support from their other family members. So, the unemployed patients might report higher levels of fatigue.

In the present study, participants living with their spouse and children or only their spouse reported less fatigue than those living singly. This finding does not surprise us. This result may be explained by the nature of Turkish culture where all patients and caregivers live with their partners, and children. Almost all families in Turkey are in close contact, and most Turkish people value family intimacy and family cohesiveness. As stated by Aştı et al. (2006) the 'family first' ideology might have motivated family members to help to maintain and improve the health of their family member (18). There is a strong tradition in Turkish culture of respecting and protecting the family members. If a family member has a chronic disease such as end stage renal disease or cancer, the spouse or children may often be in the position of being the first to help the patient, and they also may play a role in decreasing stress and helping recovery. Therefore, participants living with their spouse or children were less fatigued than those living singly.

There were no significant mean differences in fatigue scores between male and female respondents in the present study, although females reported higher fatigue scores. Similarly, McCann & Boore (2000) reported that no significant difference was found between the fatigue scores of males and females (6). Bonner et al. (2008) also reported that females experienced more fatigue than males, but this difference was not significant (5). These results are unlike those of Brunier and Graydon (1993) who reported higher fatigue scores for men and those of Q'Sullivan and McCarthy (2007) and Liu (2006) who established females as experiencing significantly more fatigue than males (13,7,4). Among the reasons why the fatigue score in female patients may be more severe than in male patients is that, in Turkish culture, female participants can articulate their feelings more than males. In Turkish

society men perceive the ill state as a loss of their power. Therefore men may be unable or unwilling to respond to symptom experience. Additionally, in Turkish society, the responsibilities of women within the home are greater than those of men, which may be another reason why their fatigue level is higher than men's. Mittal et al (2001) also suggest that gender differences may be attributable to biological factors and cultural conditions (19).

Liu (2006) reported that fatigue scores did not differ between any possible pairs of educational levels. This result is consistent with the findings of the present study (4).

Most patients who had any chronic disease, particularly end stage renal disease, tend to limit their activities during treatment. They may do so as a way to conserve their energy in order to cope with fatigue. In Turkish society, having a chronic disease diagnosis such as cancer or ESRD may make individuals more passive. This sort of thinking may also cause patients to decrease their activities and increase their tendency to rest. Or perhaps, subjects with higher frequencies of uncomfortable symptoms of fatigue might lack the energy to endure the requirements of a job. Thus, unemployed participants might report higher levels of fatigue.

Relationships between fatigue and some characteristics

Sklar et al. (1996) found that age had no impact on fatigue reported by renal patients (20). The findings obtained in the present study do not support this. However, Liu (2006) established that age was identified as a factor associated with fatigue in patients on hemodialysis in Taiwan (4). This result is consistent with the findings of the present study.

Cardenas and Kurtner (1982) found that duration of dialysis is a factor that positively correlates with fatigue (21). This result is in contrast with the findings of the present study. However, the data of the present study are consistent with other studies (22,6). It can be seen that contradictory results were found concerning correlation fatigue and duration of hemodialysis. We suggest that further research is needed in the future.

Limitations of this study

This study has some limitations. Firstly, we enrolled only HD patients. This may mean that the results of the study are not generalizable to patients receiving hemodialysis or peritoneal dialysis. It reflects only one area of Turkey. It is also acknowledged that the findings may be culturally specific, although the tool used has been evaluated both in Turkey and other settings for its reliability and validity. There is scope however, for the study to be replicated in different cultural settings and a large population on hemodialysis and peritoneal dialysis than those represented in our study. Finally, in this study, physiological variables (Hb, albumin, urea reduction ration (URR), the urea kinetic equation (Kt/V),and psychological factors (depression, anxiety, sleep quality) that could be associated with fatigue in renal patients were not examined. Future studies should include these factors in Turkey and in other ethnic groups.

Conclusion

Our results indicate that fatigue in patients receiving hemodialysis treatment is highly prevalent and severe. The findings also indicate significant relationships between fatigue and marital and employment status, and living with other people. In addition other findings demonstrated that gender and educational and economic factors were not associated with the experience of fatigue in hemodialysis patients. Therefore it is essential for nurses not only to understand fatigue, its side effects and factors causing the experience of fatiguebut they also need to actively and effectively assist patients to address and cope with it.

Relevance To Clinical Practice

According to our results, it is clear that fatigue is a severe symptom experienced by patients on hemodialysis. Nurses, who play a key role in managing the fatigue symptom, can draw several implications for their practice from this study. First, nurses need to admit fatigue as a prevalent symptom in patients on hemodialysis. Additionally, they should assess patients with regard to fatigue and assist patients to develop coping strategies to prevent and respond to it. Second, the PFS is a simple tool that can be used in a clinical setting and therefore it could be used to assess patients' subjective perception of fatigue as a regular routine. Lastly, practical strategies for managing fatigue in patients receiving hemodialysis as well as those receiving cancer-related fatigue management

need to be developed, implemented and evaluated. The strategies developed for managing fatigue in other chronic disease groups should be tested in patients on hemodialysis to determine their applicability.

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The effect of approaches that are carried out in children to whom catheter is inserted and their parents on children's pain and parent's anxiety

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Abstract

Aim: The study was performed as a semi-experimental model to determine the effect of nursing practices on pain in children and parental anxiety during urethral catheterization.

Materials and methods: The study was conducted between October 2010 and April 2011 at department of pediatric surgery, GMMA. The data was obtained from children and their parents at the outpatient clinic. Quota sampling was used and 44 children met the study criteria and agreed to participate in the study. The Children were divided into two equal groups. In this study, data collection of the descriptive properties of parents and their children, Spielberger state-trait anxiety inventory, Face pain rating scale (Wong- Baker), Physiological parameters of children before and after operation follow-up form, children and their parents operating reveals behavioral observation form were used for data collection.

Results: There was a statistically significant difference between the control and the study group when compared for the pain and the parental anxiety.

Conclusions: The nursing practices have very significant impact to reduce both pain in children and parental anxiety during the urethral catheterization

Key words: Urethral catheter, children, parents, pain, anxiety, nursing approaches

Introduction

It's important to manage pain effectively in childhood. The first experiences of pain affect the reaction of the child towards painful procedures and conditions in the future; and if their experiences are negative, some physiological and psychological problems like forming changes in the pain sensitivity lifelong may be established (1-3).

As well as providing the urine drainage that the child who has genitourinary problem may come across a lot; urethral catheterization is also an invasive procedure that is unpleasant, that creates pain and anxiety, and that has physical, mental and social consequences (1,3). It is indicated that children may feel pain and shame during urethral catheterization because of reasons like being naked on the examination couch and their genital organ being examined by a stranger, an unknown device being inserted into their body and having to urinate before other people (4).

It should be considered that pain in children is a subjective perception which is dependent on environmental and individual factors. Pain in children is measured by listening to the individual statements of the child, observing his/her behavior and using the physiological measurements according to his/her age and cooperation; and the individual statements of the child is accepted as the gold standard (2, 5-7). In order to provide the appropriate pain care, nurses should know how to detect the pain. Various measures are developed to help defining the intensity of the pain. The most ideal evaluation tools are the measures that are appropriate for the age of the child and in that both the nurses observe the behavior of the child and the child can report the pain (2, 8-11).

The reaction of the child towards the procedure and the pain originated from the procedure may increase if the child is not informed about the painful procedures and if the people who the child trusts are not with him/her (12). Parents have a unique role in children's pain management: Usu-

ally they know more than anyone else about their child's past pain experiences, their coping style, their interests and their fears. Consequently, many parents wish to stay with and comfort their child during a painful procedure and are frequently asked by their child to do so. However, some parents are highly anxious about entering the treatment room and about their own ability to support their child through a painful experience, and as a result may choose to avoid being present (13, 14).

In the pain management that is carried out with multidisciplinary approach, nurses have active roles that are dependent on the reasons like being with the patient for a longer time, learning the previous pain experiences of the patient and the ways to cope with them, making use of these when needed, teaching the patient the strategies to deal with the pain, guiding the patient and monitoring the results of the procedure (15, 16).

Material and Methods

This study was done as a quasi experimental and descriptive one for the purpose of evaluating the effect of nursing practices on pain in children and parental anxiety during urethral catheterization.

The sample of the study is composed of 5-12 age group children and their families who will have urethral catheterization during September 2010- April 2011 in a Child Surgery Department Policlinic in an education and research hospital and who accept to attend the study. In the study, 22 children with their parents were chosen as experimental group; 22 children and their parents were chosen as the control group by doing randomization to the children and the parents by sealed tender. Approval was obtained from the ethics committee of the Gülhane Military Medical Academy.

In this study, two questionnaires including socio-demographic features of the child and parent; an evaluation form for physiological parameters of children (blood pressure, pulse and oxygen saturation) before and after the procedure; an observation form on which the behaviour of the children and parents during the procedure is recorded; Spielberger's state anxiety inventory (17) that evaluates parents' anxiety and Wong-Baker Faces Rating Scale that is used for the pain diagnosis of children at the age of 3 and older (2,18-21). The scale is a self-assessment tool. Nurses who use the scale ask children to point at the face on the scale that best represents how they feel about their pain. Parents and children who met the inclusion criteria received oral information about the study from a nurse.

What the parents and children know about the urethral catheterization procedure is learnt, their lack of information is removed, and they are guided for what they wonder about the procedure. During the procedure, non pharmacologic methods were shown to the children and their parents. In the training, an information brochure prepared by the researcher, pictures that include the steps of the procedure and materials for the procedure are used.

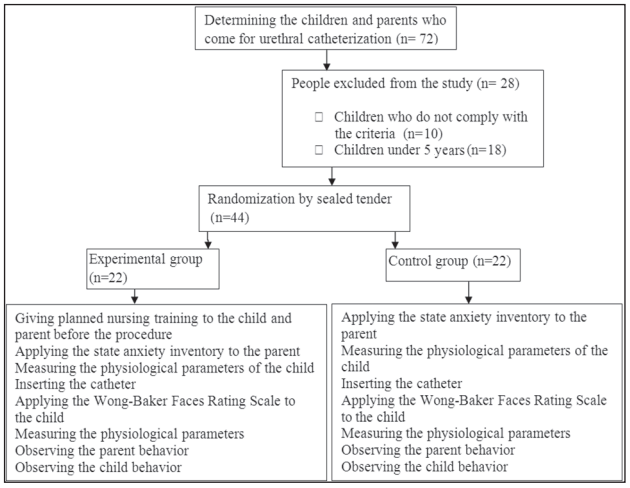

Figure 1. Data Collection Procedure

Statistical Analysis

All statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago,IL). Mann-Whitney U test was applied in order to find the difference between the two groups' data determined by the measurement. In groups more than two, intergroup differences were compared by Kruskal-Walis test. When comparing the difference between two percentages, chi-square test was used. In statistical decisions, $p<0,05$ level was accepted as the indicator of meaningful difference.

Results

There was no statistically meaningful difference found between the groups in terms of defining characteristics ($p>0.05$) (Table 1)

Table 1. Distribution of defining characteristics about children's socio-demographic and illness (N=44)

Demographic characteristics	Experimental group (n=22)	Control group (n=22)	Test*	p**
	n (%)	n (%)		
Gender				
Girl	15 (68.2)	12 (54.5)	0,863	0,353
Boy	7 (31.8)	10 (45.5)		
Age				
5-6 age	14 (63.6)	14 (63.6)	0,000	1,000
7-12 age	8 (36.4)	8 (36.4)		
Average of age	6,7 (min:5-max:12)	6.5 (min:5-max:10)		
Diagnosis				
Urethral anomalies	-	1 (4.5)	1,599	0,450
Urinary bladder anomalies	14 (63.6)	12 (54.5)		
Ureter anomalies	8 (36.4)	9 (40.9)		
Having Been Catheterized Before or Not				
Yes	17 (77.3)	16 (72.7)	0,121	0,728
No	5 (22.7)	6 (27.3)		
Having Entered Into a Hospital Before or Not				
Yes	15 (68.2)	14 (63.6)	0,101	0,750
No	7 (31.8)	8 (36.4)		
Having Had Painful Procedure Before or Not				
Yes	17 (77.3)	18 (63.6)	0,140	0,708
No	5 (22.7)	4 (36.4)		
Person who is with the child during the procedure				
Mother	10 (45.5)	10 (45.5)	0,202	0,904
Father	4 (18.2)	3 (13.6)		
Mother- Father	8 (36.4)	9 (40.9)		

**χ^2= Chi- square test ** p>0,05*

Table 2. Comparing the pain scores of the children in the experimental and control groups

Pain Scale Score	Experimental Group	Control Group	Test*	p**
	$\bar{X} \pm S$	$\bar{X} \pm S$		
	2,09 ± 1,15 (min-max=0-5)	3,09 ± 1,65 (min-max=0-5)	-2,039	**0,041**

**z = Mann Whitney U test ** p<0,05*

In our study; according to the Wong-Baker Faces Rating Scale, pain score average of the children in the control group is higher than the pain score average of the children in the experimental group, and the difference between them is found to be statistically meaningful (p<0,05) (Table 2)

In figure 2, the pain levels that the children in the experimental and control groups expressed in Wong-Baker Faces Rating Scale are given. Most of the children in the experimental group (36.3 %) expressed that they had 'little more pain' after the procedure; whereas most of the children in the control group (27.2 %) expressed that they had 'the worst pain'.

In our study; most of the parents in both groups are the mothers (EG: 72.7%; CG: 77.3%) and they have experienced painful procedure with their child before. There was no statistically meaningful difference found (p>0,05) among the parents in the experimental and control groups in terms of defining characteristics.

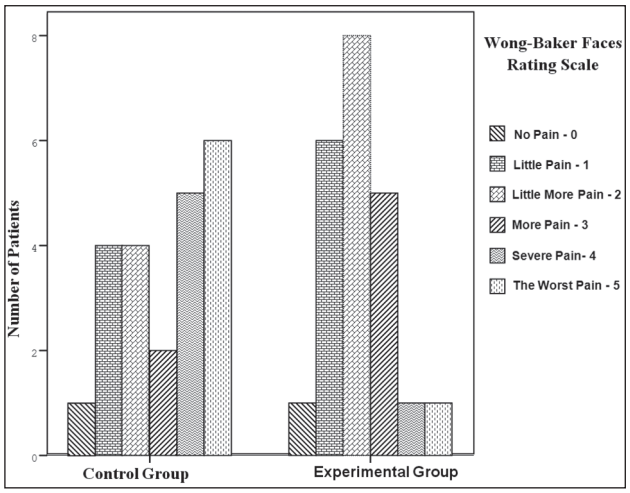

Figure 2. Distribution of the pain levels of children in the experimental and control groups

When the anxiety states of the parents in the experimental and control groups before the procedure are compared, it was found that the difference between the groups was statistically meaningful ($p<0.05$) (Table 3).

During the procedure parents demonstrated positive behavior: 95.5% of the parents tried to attract their child's attention to something else; 90.4% of them provided physical support; and 86.4% of them said sympathetic words to their children. It is determined that there was a statistically meaningful difference ($p<0.05$) among the parents in the experimental and control groups in terms of attracting the attention to something else; in terms of other behavior, there was not any statistically meaningful difference among the two groups.

Children's behavior of coping with pain is shown in Table 4.

During the procedure; children who demonstrate positive coping behavior like concentrating on something else, relaxing, attending the procedure, and hugging have lower pain score averages than the children who do not demonstrate these behavior and the difference between them was found to be statistically meaningful ($p<0,005$) (Table 5).

It was recorded that after the procedure, physiological parameters like oxygen saturation, diastolic blood pressure, and systolic blood pressure values of the children in the experimental and control groups are decreased; whereas their heart rate is increased. It was found that there was no statistically meaningful difference between the groups ($p>0,05$)

Table 3. Comparing the anxiety states of the parents in the experimental and control groups before the procedure

States of Anxiety	Experimental Group (n=22)	Control Group (n=22)	Test*	p**
	n (%)	n (%)		
Mild Anxiety	8 (36.4)	8 (36.4)	6,890	**0,032**
Moderate Anxiety	13 (59.1)	7 (31.8)		
Severe Anxiety	1 (4.5)	7 (31.8)		

*χ^2 = Chi- square test ** $p<0,05$

Table 4. Comparing the behavior demonstrated by the children in the experimental and control groups during the procedure

Behavior of Children	Experimental Group (n=22)	Control Group (n=22)	Test*	p
	n (%)	n (%)		
Attending the procedure	17 (77.3)	5 (22.7)	13,091	**0,000****
Concentrating on Something Else	16 (72.7)	8 (36.4)	5,867	**0,015****
Relaxing	12 (54.5)	3 (13.6)	8,193	**0,004****
Hugging	10 (45.5)	4 (18.2)	3,771	**0,052****
Expressing the Pain Orally	6 (27.3)	5 (22.7)	0,121	0,728
Yelling, Crying, Groaning	5 (22.7)	15 (68.2)	9,167	**0,002****
Resisting	1 (4.5)	12 (54.5)	13,211	**0,000****

*χ^2 = Chi- square test ** $p< 0,05$

Table 5. Comparing the children's behavior with pain score state

Behavior of Children	Pain Scores $\bar{x} \pm s$	Test*	p
Concentrating on Something Else			
Yes	1,66 ± 1,04	-4,516	**0,000****
No	3,70 ± 1,17		
Relaxing			
Yes	1,53 ± 1,18	-3,479	**0,000****
No	2,13 ± 1,35		
Attending the procedure			
Yes	1,72 ± 1,07	-3,778	**0,000****
No	3,45 ± 1,01		
Hugging			
Yes	1,71± 1,25	-2,755	**0,006****
No	3,00± 1,46		
Resisting/ Kicking			
Yes	4,23± 0,72	-4,613	**0,000****
No	1,90± 1,16		
Yelling/ Crying			
Yes	3,75± 1,11	-4,745	**0,000****
No	1,62± 1,01		

** z= Mann Whitney U test ** p<0,05*

Discussion

Children may come across with painful procedures anytime in their lives for the purpose of diagnosis or treatment. Research has found long-term traumatic memories for some children receiving painful medical procedures. Furthermore, "… an intervention that successfully reduces children's negative memories may alleviate their distress during future stressful events" (22, 23). Children may have anxiety being in the hospital environment and coming across with an unfamiliar environment, unfamiliar people, especially people with uniform, medical equipment or stress of the parents (24). In the study, it is thought that the training given before the procedure to the children in the experimental group explaining why the urethral catheterization will be done, who will do the procedure, how much pain they will feel, how to deal with the pain, and whether their parents will be with them during the procedure or not has a positive effect on children to perceive their pain and cause them to feel less pain compared to the control group. It is indicated in the literature that children who state that they do not know why the painful procedure is done have lower pain tolerance, whereas the children who know that the procedure is done in order to find out the illness demonstrate the highest pain tolerance (6) (Table 2).

The Accreditation Commission of the Health Agencies (JCAHO) has specified four needed standards related to pain management. The statements: 'pain of all the patients should be considered' and 'the patients have the right of pain management' are only two of the standards. The assessment should be convenient with the age of the child, general situation, the level of perceiving the pain, and his/her skills. Perception of pain is a subjective situation. That is why; the individual's own statement is the most dependable and the most acceptable method of pain evaluation especially in the school age and adolescence (5, 25). Because most of the children who are 3 years old and above can express their pain and the level of their pain; it is possible to ask them to show their pain level choosing from a series of colors or pictures (2, 20). In our study, it is thought that giving a planned training to the children in the experimental group about the procedure beforehand, guiding them about how to behave during the procedure and parents using the helping approaches during the

procedure help children to be relaxed during the procedure, help them feel less pain and thus they express low level pain Wong-Baker Faces Rating Scale which is a self-evaluation scale (Figure 1).

In our country, traditionally, the needs of the child are mostly met by the mother and in case of an illness or in situations like dealing with painful procedures, mothers mostly take the responsibility of care. In our study, the facts that the parents in the experimental and control groups do not have any statistical difference between them in terms of defining characteristics, and that in both groups, parents are mostly the mothers, show that the study groups are similar in terms of the variables determined for the study groups.

During the painful procedure, parents' thoughts about not being able to help their children, not being able to protect them from the painful procedures, losing the control of making decisions in the face of the procedures done, the unfamiliarity of the hospital environment and sanitarians using medical terminology are among the important reasons of anxiety (26). In order to decrease the anxiety created, parents should be provided with some anxiety lowering approaches like informing the parents about the procedure and showing them the behavior types during the procedure. Being informed provides control over the situation (27, 28). In our study, giving an informative brochure that explains the procedure with pictures to the parents and children in the experimental group and providing training with the materials used in the procedure decreases the anxiety level and the anxiety- creating reasons; that is why it is thought that they have a lower anxiety level than the parents in the control group (Table 3).

Families mostly want to stay with their children during medical procedures; but they do not know what to tell their children, how to behave, and what to do to make their children deal with the pain. Recent politics suggest that parents be with their children during their acute pain experiences. Educating the parents about the child's pain management helps them to feel sufficient and useful as well as decreasing the anxiety of the child (6,14, 26, 28). There are some non-pharmacological methods to help the children and his/her parents during the painful procedures like attracting the attention to something else, attending the procedure and relaxing. These methods should be convenient with the age of the child and individualized. The behavior of attracting the attention to something else helps to concentrate on another stimulus rather than the pain and thus increasing the pain tolerance and decreasing the pain sensitivity. This is especially helpful in short- period invasive painful procedures (6,15,29,30). In our study, it is thought that the planned nursing training given to the parents in the experimental group in order to deal with the pain of the children during the procedure is effective for the parents to use the behavior of 'attracting the attention to something else' more.

In literature, it is indicated that increase of the stress behavior of the child and parent's physical support approach are related to each other (6, 26, 31). In our study relaxing the child and decreasing the stress is aimed through behaviors like holding the child's hand, kissing him/her, and caress him/her as the physical support behavior. Most of the parents in the experimental group (90.4%) use the behavior of physically supporting their child in comparison with the parents in the control group (77.3%).

Using positive and ego-supportive statements are the inseparable parts of managing the stressful procedures (32). Children who are exposed to embarrassing statements by their parents tolerate the pain less (30, 33). In our study, it was observed that just one parent in the control group uttered some statements like 'remove your hand, this is not the first time it is inserted to you, you always do the same thing, men do not cry'. It is thought that the reason why just one parent uttered embarrassing statements and other parents did not have such an approach is that most of the parents (50%) have an undergraduate level degree and thus their attitude was not judging their child.

Pain is an individual experience related to behavioral, emotional, physiological, socio-cultural and developmental factors and its management is founded on individual evaluation (2, 6). Gaining children's confidence, enabling them to express themselves by giving correct explanations, including them in the care, and teaching them the ways to deal with the pain are important approaches. In literature, it is stated that most of the children decrease their pain by relaxing, taking the correct position, attending the procedure, and concentrating

on other things during the procedure (6,12,32,33). It is thought in our study that the reason why the children in the experimental group demonstrate the positive dealing behavior related to decreasing the pain like concentrating on something else, relaxing, attending the procedure and hugging more than the children in the control group is; the nurse showing them these approaches before the procedure as well as the parents approaching them positively (Table 4).

It is stated in the literature that children who use the positive dealing methods during the painful procedures are more relaxed, deal with his/her pain better, and have lower pain scores (34,35). Hugging is one of the comforts in the childhood, it is used frequently in stressful and painful situations, it restricts the child's moves, and it is a positive and relaxing action (32). In our study, it is identified that children who demonstrate positive dealing behaviors like concentrating on something else, relaxing, hugging, attending the procedure, and receiving physical support during the procedure experience the pain less and have low pain score averages; and statistically meaningful difference was found between them and the children who do not demonstrate positive dealing behavior ($p<0,05$) (Table 5). Pain initiates a stress response triggering cardio respiratory and hormonal/ metabolic changes. Heart rate, blood pressure, and changes in the respiration are a result of a frequently increasing sympathetic activity that can be related to pain and stress. In literature, among the physiological measurements, heart rate is the most frequently used one; and there are a few studies in which the other physiological measurements are used (5,9,11). In our study, the fact that only the heart rate increased and the other physiological parameters are decreased after the procedure is the indicator of the compensator mechanisms and it is observed the same way in both groups.

Conclusions

The training given to children about urethral catheterization and the guiding approach ensures the children to deal with the pain better and feel less pain. It was observed that the nursing approach and training applied to the parents before the procedure help decreasing the anxiety situations. The non- pharmacological methods the parents use during urethral catheterization like attracting the child's attention to something else, providing physical and emotional support, and praising help the child to adjust to the procedure deal with the pain positively. It was found that children who demonstrate positive dealing behavior to deal with the pain during the procedure have lower pain score averages. It was also found that the physiological parameters taken before and after the urethral catheterization is not efficient alone.

Showing non-pharmacological methods to children and parents before procedures with anxiety and pain like urethral catheterization, using visual materials in the training given, communicating with the child and parents during the procedure, and continuing the communication in a way that will weaken the child's pain should be the standard part of the nursing applications. It is suggested that the studies to be done in larger sampling groups in order to generalize the results of the nursing approaches done to the society related to removing the pain and anxiety of the children and parents that the urethral catheterization is applied.

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The nursing care of severe brain injury patients with septic shock

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Abstract

Objectives: To explore the observation and nursing care of patients suffered from severe traumatic brain injury complicated with septic shock.

Methods: To analyze 32 patients with severe traumatic brain injury complicated with septic shock in Shanghai Neurosurgical Emergency center and Worldwide Medical Center of Huashan Hospital from June 2008 to June 2009 retrospectively. We summed up the causes of septic shock and relevant nursing care measures.

Results: All the patients received positive therapy, care and nursing, and followed up for 3-6 months. 31 patients survived and 1 patient died.

Conclusions: It is important to monitor such patients comprehensively and carefully. Early diagnosis of shock, reasonable volume resuscitation, proper usage of vasoactive drugs, and other related basic nursing care could help to increase the survival rate and decrease the morbidity.

Key words: Severe traumatic brain injury, septic shock, observation, nursing care.

Introduction

Severe traumatic brain injury is defined as Glasgow Coma Scale (GCS) ≦8[1] after brain injury. The patients with severe brain injury are serious. They usually lie in bed for long time, and the environment inside the body is disorganized. Thus, it is easy to be malnutrition and immunocompromised. During rescuing them, most of the patients need to be monitored the intracranial pressure, tracheotomy for ventilation, detain of deep vein catheter and retention of urinary catheterization. Sometimes continuous external lumbar drainage is necessary. Such invasive manipulations make patients vulnerable to infection and severe patients may affiliate infective shock. Therefore, it is troublesome to treat and nurse such kind of patients, and lead to poor prognosis. However, how to treat and nurse these patients is quite important. In this study, we retrospectively summarized the nursing care of 32 patients who suffered from severe brain injure with septic shock, and provided basic information for formulating related measures of clinical nursing care.

Methods and materials

Subjects: From June 2008 to June 2009, 32 cases of severe brain injury with infective shock treated in Shanghai Neurosurgical Emergency center and Worldwide Medical Center of Huashan Hospital were selected. There were 20 males and 12 females. The age range was 21-78 years old and the average age was 42.4 years old.

Treatments: Controlling the source of infection after resuscitation as early as possible. Carrying out the following treatments such as intravenous antibiotics, hemodynamics support, mechanical ventilation, blood transfusion related treatment, appropriate sedative and analgesia therapy, blood sugar control, kidney function protection, prevention and supportive treatment of stress ulcer.

Methods

Careful observation and early judgment of shock: The patients with severe brain injury were in coma, so it is more difficult to judge shock than the conscious ones. Thus, clinical manifestation and laboratory examination were combined to judge the situation.

Observation of body temperature and consciousness: If the body temperature is not upgrade or fluctuated between 39℃-41℃, and the consciousness of patients aggravate, then the shock occur. In all patients, 31 patients' body temperatures fluctuated between 39℃-41℃ in the initial stage of shock, while 1 patients' body temperature did not elevate.

Catheter related blood stream infection (CRBSI): There were 8 patients with central venous catheter occurred subinfection among the 32 cases in our study.

Pulmonary infection: The patients with severe brain injury and coma have weak cough and swallowing reflex, thus they are risk of aspiration due to sputum accumulation in the throat. 30 patients in our group were performed tracheotomy.

Specific laboratory test: The determination of lactic acid: the normal value of lactic acid is 2 mmol/L. The value over 4 mmol/L indicates unfavourable prognosis. If the value decreased quickly, the prognosis would turnover well. In our study, the lactic acid value of 31 patients was 2.2-4 mmol/L, and 1 patient 3.5-4.9 mmol/L.

The determination of C-reactive protein (CRP): the normal value of CRP is ≤10mg/L. If the value over 10mg/L, it indicates infection in the body. The higher is the value of CRP, the worse is the patient's condition and prognosis.

Results

The results of etiology: There were 18 cases of shock secondary to the pulmonary infection, 3 cases of intracranial infection, 3 cases of urinary system infection, 8 cases of catheter related septicemia (Table 1).

Table 1. The secondary source of septic shock

The secondary source of shock	Case number	Percentage
Pulmonary	18	56.4%
Intracranial	3	9.3%
Urinary system	3	9.3%
Catheter related septicemia	8	25%
Total	32	100%

The hemoculture of bacteria and bacterial smear revealed that there were 21 cases infected with Gram-negative bacteria, 5 cases infected with Gram-positive bacteria, 6 cases infected with mixed bacteria, and 8 cases infected with fungus among the 32 patients (Table 2).

Prognosis: Through effective treatment and careful nursing, 31 patients in our study got better, 1 patient was stable and discharged because of financial problem. The average hospitalization was 26 days. The score of prognosis GOS was 8-12. 3-6 months follow-up showed 31 patients were alive (96.9%), and 1 patient died (3.1%).

Table 2. The hemoculture and bacterial smear

Name	Case	Combined fungus infection
Gram-negative bacteria	21	5
Gram-positive bacteria	5	1
Mixed bacteria	6	2
total	32	8

Discussions

The nursing during volume resuscitation and vasoactive drug usage

The nursing of volume resuscitation: The rate and volume of transfusion is dependent on the blood pressure, central venous pressure, urine volume, heart rate, heart sound, respiration, the patients' clinical manifestation and the patients' reaction to volume resuscitation. The resuscitation goal should be achieved within 6 hours: central venous pressure is 8-12cmH_2O, mean arterial blood pressure ≥ 65mmHg, urine volume ≥ 0.5ml/kg.h[2]. In the study, we gave the patients quick and sufficient transfusion at early stage if the septic shock occurred. Once the resuscitation goal was achieved, the rate and volume of transfusion decreased quickly. When the transfusion rate decreased, clinical index of the patient such as arterial pressure, pulse rate, pulse pressure, peripheral circulation status, skin color, urine volume and central venous pressure should be kept normal. Because it would significantly increased the risk of pulmonary edema when central venous pressure is over 15-18cmH_2O[3]. If the transfusion rate decreased, the patient showed blood pressure dropping, heart rate increasing, sweating profusely, breathing rapidly, irritability, and the clinical manifestation would get better after speeding up transfusion. Then the volume resuscitation is safe as long as the patient is carefully nursed.

The nursing of using vasoactive drugs: If the vasoactive drugs are used, the blood pressure should be monitor to change the transfusion speed. When the blood pressure is stable, the vasoactive drugs should be removed gradually and the blood pressure should be monitor. In our study, all the patients were cared by ECG monitoring, the blood pressure was measured every 15-30minutes, and the pump was used to deliver the vasoactive drugs.

Mechanical ventilation and pulmonary infection care

Mechanical ventilation: ALI/ARDS patients' goal ventilation volume were set by weight/kg (6ml/kg), and protective mechanical ventilation required target platform pressure less than 30cm-H_2O[4]. The PEEP was set in order to avoid alveolar collapse. In our study, the ventilators were used for about 29.6~456h with average time of 124h.

Nursing care of pulmonary infection: Patients with severe brain injury were usually coma. The sputum was easily occluded in the throat and induced aspiration because of weak cough and swallow reflex. It should strengthen phlegm sucking, and the tracheotomy should be performed as early as possible. In our study, 30 patients were performed tracheotomy. For the patients with tracheotomy, it is important to grasp the sterile phlegm sucking skill, clean the respiratory secretion in time, knock over the patient at regular time, moist the airways, promote the sputum discharge, and keep airways open. For some patients with severe pulmonary infection, it is necessary to suck sputum by fiberoptic bronchoscopy. The phlegmy bacteria and fungi smear should be performed regularly in order to guide the use of antibiotics.

Intracranial infection nursing: 3 patients in our study showed high temperature, headache, and neck stiffness. The nurses should inform the doctor quickly, give the patient antibiotics as the doctor prescribed, and help the doctor to perform lumbar puncture drainage and sheath antibiotics injection. The ward should be kept clean, disinfected and declined to visit.

Nursing care of urinary tract infection: The coma patients need long-term urethral catheter, thus it is easy to induce urinary tract infection. The nurses should keep the urinary catheter unobstructed, place the urinary catheter and urine bags below the loin area to avoid urine reflux, close the urinary catheter for a period of time to practice the bladder function, clean perineal area, nursing the urethra daily, and take bladder washout if necessary. The urine test should be performed every week, the urine color, properties and volume should be monitor.

Nursing of deep vein thrombosis prevention: Strengthen patient turning back, raise lower extremities and move lower limbs in time, use DanLiMo or intermittent lasting air pressure[5], encourage patients to take deep breath in order to reduce the risk of vein thrombosis.

The basic nursing care

Oral nursing: Use the saline tampon to wipe the oral cavity 2-3 times every day. It should not be too much water for the coma patients in case of suffocating when wiping. The nurses should carefully observe the oral mucous when wiping, if there was fungus infection, local drug such as mould element glycerin could be used, or adjusting the gargle fluid according to swabs culture results.

Skin care: Brain injury patients with septic shock were in coma for a long time, no active movement, in fever and sweating, thus the long-time pressed body parts were prone to induce pressure sore. It is important to perform the skin care for such kind of patients. Turn the patients in time, keep the bed clean and dry, and use bed mattress.

Others

High fever care: Patients with severe brain injury were in bed for a very long time, and they were in a status of high metabolism, high decomposition, and low immunity. High fever could increase the whole body metabolism especially brain metabolism. If the body temperature elevates 1 ℃, brain metabolism would increase by 13% [6], and brain tissue was in relative low oxygen, which would aggravate the brain damage, hence it needs to take active hypothermia measures, such as physical cooling including ice, ice cap or ice packs over head and neck, axilla, groin, etc; wipe the whole body with alcohol; use drugs to cool the body, such as anti-inflammatory pain bolt; or even hibernate therapy. If the patients with severe brain injury got high fever, rapid respiration and tachycardia would be onset. Therefore, it needs to reverse the patient's temperature back to physiological condition in shortest time, which could decrease the patients' metabolic rate and reduce oxygen consumption. It would contribute to the improvement of hemodynamics and respiratory condition. At the moment, the most quick and effective cooling measure is physiological hypothermia.

Prevention of stress ulcer: Stress ulcer is mainly caused by damage to the nervous pathway of hypothalamus parasympathetic center and medulla

oblongata center during brain injury. The parasympathetic nerves without inhibition excite, cause excessive secretion of hydrochloric acid, and lead to extensive erosion and hemorrhage of gastric mucous [7]. Meanwhile, the body would ensure the blood perfusion for important organs such as heart and brain during septic shock at the expense of decreasing the blood supply for internal organs such as stomach and intestine, which lead to further ischemia of gastric mucous. In our group, when the patients were resuscitation and the hemodynamics was stable, we gave the patients gastric tube at early stage, applied protective agents for gastric mucous, provided small dose of continuous nasogastric feeding, and monitored stomach retention and gastric juice characteristics. Due to delayed gastric emptying in such kind of patients, stomach retention should be monitor closely. The gastric tube should be clipped 1 hour before meal, and gastric contents were withdraw 1 hour after meal to record its color and volume, and the gastric juice was injected back to the stomach. If the volume of stomach retention was over 150ml, the nasogastric feeding should be suspended. Thus, monitor stomach retention would help to know the status of acute gastric mucous injury. Reflux of acid stomach contents would be aspirated to lungs, and cause trachea spasms and aspiration pneumonia.

Peripheral blood sugar monitor: Blood sugar increase is the results of stress reaction during brain injury [8]. The extent of blood sugar increase is positively related to the severity of brain injury and prognosis. For severe brain injury patients, if blood sugar is over 11mmol/L after injury, the prognosis is obviously poorer than the patients with blood sugar less than 11mmol/L. The patients with septic shock could also appear hyperglycemia due to the excessive release of catecholamine. To those patients with septic shock difficult to remedy, the usage of corticosteroids could aggravate stress hyperglycemia. Therefore, it is extremely important to monitor blood glucose and control its level in severe brain injury patients with septic shock. Hyperglycemia could not only aggravate neural cells injury, but also cause abnormality of internal environment, which would aggravate secondary brain injury. In 12 patients of our study, we strengthened the monitor of peripheral blood glucose during and after resuscitation, and the blood glucose was monitored every 30 minutes. If the blood glucose was abnormally elevated, the insulin was continuously intravenously infused. The transfusion rate was dependent on the initial blood glucose level, and the blood glucose should be kept between 5-8mmol/L. If the blood glucose level is over 8mmol/L, the initial dose of insulin is 2u/h. If the blood glucose level is over 10mmol/L, the dose of insulin should be adjusted to 3u/h. If the blood glucose level is over 12mmol/L, the dose of insulin is 4u/h, and the like. The higher is the blood glucose level, the shorter is the monitor intervals. If the blood glucose is tend to be stable and close to the therapy plan. The monitor interval of blood glucose should be prolonged to every 4 hours. The transfusion kind and volume should be adjusted according to the blood glucose. 1 patient in our group was diagnosed diabetes before injury, and we must pay much more attention to the deadly hyperglycemia in the patient, which would result in hyperosmotic nonketo acidosis, in order to achieve satisfactory therapy expectation.

Conclusions

Septic shock is a serious complication of severe brain injury. Its pathogenesis is complex, the fatality rate is high, and the treatment is difficult. Therefore, the nurses need to observe patients seriously, promptly and exactly. The nursing care should be careful, thoughtful and overall[9]. Judge the shock as early as possible, nursing when blood volume expansion was conducted and vasoactive drug was used, monitor patients' peripheral blood sugar, and prepare proper nursing care and basic nursing to different infection, thus it would promote the recovery of severe brain injury patients with septic shock, increase the cure rate and decrease the morbidity.

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Examination of nursing students' attitudes towards older people in Turkey

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Abstract

Purpose: This study aimed to examine the attitudes of nursing students toward studying at Nursing Faculty of Dokuz Eylül University as well as the relationship between their personal characteristics and attitudes.

Methods: This is a descriptive and correlational study and was performed on 417 students studying at the nursing faculty. Data were collected using a sociodemographic characteristics questionnaire and the Kogan's Attitudes Towards the Old People Scale. Variance, significance of difference between two averages test and Kruskall Wallis tests were employed according to the data characteristics.

Results: When score averages of students obtained from Kogan'a scale were examined, positive score average was found as 61,22 ± 14,79 and negative score average was found as 79,98 ± 13,62 while the overall score average was found as 154,73 ± 22,08. Score averages obtained by the first, second, third and fourth class students from Kogan's scale were determined as 145,31 (20,81); 152,29 (18,37); 158,38 (21,92) and 168,57 (22,57), respectively.

Conclusions: In accordance with the results of this study, it can be concluded that as the class of nursing students increase, their positive attitudes towards old people also increase. Students display more positive behaviors in direct proportion to the increasing age and living an old relative within the same family also affects the attitudes of students positively.

Key words: Nursing students, attitudes, older people.

Introduction

With the improvement observed in the health and social fields in the last 25-30 years in developed and developing countries, life expectancy prolonged and this caused an increase in the elderly population throughout the world. In Turkey, elderly population is also on the increase. While the ratio of individuals aged 65 and over is 6,8 % in the whole population according to the data of Turkish Statistical Institute (2008), the State Planning Organisation declares (2007) that this ratio is expected to reach to 8.46 % by 2020 (SPO, 2007).

Such changes as physical failure, chronic disease, pain and cognitive disorders which appear with the prolongation of lifespan are important health problems affecting the elderly individuals, their families and the health system negatively. Nurses have a fundamental role in preventing and delaying these problems or ensuring the elderly individuals to cope with such problems. Thus, nursing educators should be aware of the demographic changes and provide their students who will undertake the health services in the future with the necessary competencies to keep up with the time.

Attitudes of students towards elderly affect their decisions of working with them as well as their care behaviors (Cortney et al., 2000). Therefore, nursing students should display positive attitudes towards elderliness. Sabates and Captavilla (2010) reports that attitude has three dimensions as cognitive, affective and behavioral and these three dimensions and attitude interact constantly. Within this framework, it can be argued that a nursing training targeting changes in cognitive, affective and behavioral dimensions is one of the basic factors affecting the attitude towards elderliness. To increase efficiency of the current nursing curriculum in this respect, it is of importance to know the attitudes of students towards the elderly people. In our country, the studies examining the attitudes of students towards elderly people are limited. It is thought that knowing the attitudes of nursing students who are expected to carry out the health services in the future will be a data source for the efforts aimed at improving the nursing curriculum.

Literature Review

In the literature related to the attitudes of nursing students towards the elderly, the relationship between student characteristics and attitude was examined. Student characteristics addressed in the study were age (Sheffler 1998; Mosher-Ashley and Pamilee 1999; McCracken et al 1995; Söderhamn et a.l 2001; Stewart et al (2005; Hweidi, Obeizad 2006), gender (McCracken et al 1995; Sheffler (1998; Söderhamn et a.l 2001; Hweidi, Obeizad 2006; Zambrini et al. 2008; Wang et al. 2009), content of the nursing training/courses-practices related to the elderliness ((Haight et al. 1994; McCracken et al 1995; Shoemake and Bowman 1998; Söderhamn et a.l 2001; Stewart et al. 2005; Hweidi, Obeizad 2006; Williams et al 2007; Holroyd et al. 2009; Lambrinou et al 2009; Wang et al. 2009), previous experiences or living together with the elderly (McCracken et al 1995; Sheffler 1998; Shoemake, Bowman 1998; Mosher-Ashley and Pamilee 1999; Hweidi, Obeizad), aylik gelir, yaşadiği yer; şehir/kirsal (Hweidi, Obeizad 2006) and preference of working in the elderly clinics after graduation (McCracken et al 1995; Fox and Wold 1996; Happell 1999).

It is seen that results related to the effect of age on the attitude of nursing students towards the elderly are different. Sheffler (1999) and Mosher-Ashley and Pamilee (1999) reported that there was no relationship between attitudes of students towards the elderly and the variable of age. However, in most of the studies where age of students was defined as effective on their attitudes towards the elderly, positive attitude scores increased with the increasing age. McCracken et al. (1995) detected a positive correlation between the age of American students and their attitudes towards the elderly. Later on, Stewart et al. (2005) indicated that positive attitudes increased in individuals aged 25 and over while Hweidi, Obeizad (2006) determined that there was a positive correlation between age and attitude. Differently from (contradictory to) the abovementioned studies, Söderhamn et al. (2001) stated that students aged below 25 had more positive attitudes towards the elderly than the other students.

It is also seen that the effect of gender on the attiudes of nursing students towards the elderly people varies by different groups. McCracken (1995) and Sheffler (1998) indicated that gender was not influential on the attitude towards the elderly. However, Hweidi, Obeizad (2006), Zambrini et al. (2008) and Wang et al. (2009) demonstrated that positive attitude scores of women were higher than those of the men. The result of the study conducted by Söderhamn et al. (2001) was different from these findings, in which men were reported to have more positive attitudes towards the elderly than the female students.

Nursing training is defined as a major factor affecting the attitude towards the elderly. Sheffler (1998) stated that the attitude of the instructor was an important factor affecting the attitudes of students positively. In another study, Stewart et al. (2005) determined lower negative scores in students accompanying an elderly person once a month. McCracken et al. (1995) detected a positive correlation beween attitudes of American students towards the elderly and the education year. Söderhamn et al. (2001) determined that the attitudes of the third-year class students were more positive than the attitudes of freshman. Two studies demonstrated that nursing education was not related to the attitudes of students towards the elderly. While Williams et al. (2007) showed that attitudes of nursing students towards elderly people were similar in the 1st and 4th classes, Hayrold et al. (2009) showed that they were similar for four years. Contrary to the results of abovementioned studies, the study carried out by Wang et al. (2009) revealed that positive attitude scores of the final year students towards the elderly were lower than the sophomore students. Haight et al. (1994) also showed that attitude scores of students caring after the critically ill elderly patients reduced.

Likewise, study results concerning the previous experiences or living with an elderly and attitudes of nursing students towards the elderly are different. McCracken et al. (1995) and Shoemake, Bowman (1998) detected a positive relationship between previous experiences with the elderly and attitudes of students. However, in the study of Sheffler (1998) and Hweidi, Obeizad (2006), attitude scores of students having past experiences were found higher than the other students but the difference was not statistically significant. In the research conducted by Mosher-Ashley and Pamilee (1999), there was no difference between students living with the elderly people or those who did not live with them.

Hweidi, Obeizad (2006) demonstrated that as the monthly income of a student increased, his/her attitude score towards the elderly decreased. In the same study, it was also determined that student living at the cities and in the rural areas had similar attitude scores (Hweidi, Obiezad, 2006). It is thought that attitudes of students towards the elderly affects their decision to work with this group. It was determined that attitude scores of nursing students towards the elderly affected their preference of working with the elderly people after graduation (Hweidi, Obeizad, 2006).

When the relevant literature is reviewed, the changeable factors affecting the attitudes of students towards the elderly and their decisions to work in the geriatrics clinics after the graduation are found to be associated with the nursing curriculum. As shown in the studies, attitudes of students were higher in the first class while they decreased in the final year (Haight et al., 1994, Wang et al., (2009). Whereas the attitudes of students were affected positively in a study including an integrated curriculum (Jansen & Morse, 2004), they were affected negatively in another study (Williams, Anderson & Day, 2007).

The first step of deciding on improvement studies in this respect is to determine the efficiency of the current situation. Thus, the research was conducted in order to examine the attitudes of nursing students studying at Nursing Faculty of Dokuz Eylül University as well as the relationship between their personal characteristics and attitudes.

Methods

Design and sample

This study was conducted between January 2012 and April 2012, on 434 students studying at the nursing faculty of a university hospital and accepting to participate in the study as a descriptive and correlational research. With the elimination of incomplete or wrong scales during the data controls, analyses were carried out on 417 (96 %) participants. Questionnaire forms were distributed to the students at the ends of courses by the researchers and were collected again by researchers after the students answered them.

Instruments

As data collecting tools, sociodemographic characteristics questionnaire form and Turkish Version of Kogan's Attitudes Towards the Old People Scale were used.

Sociodemographic characteristics questionnaire form: It was developed to obtain information about the students included in the sample and consists of questions related to the age, gender, the academic year, economical situation, home city, whether they lived with an old person previously or not, degree of the elderly that they lived together and whether they want to work with the elderly following the graduation.

Kogan'a Attitudes Towards Old People Scale: It is a likert type scale consisting of 34 items and six different answer levels following one another (Kogan, 1961a). These answers are strongly disagree, slightly disagree, disagree, agree, slightly agree and strongly agree. These categories are scored as 1, 2, 3, 4, 5, 6 and 7 respectively. Item to which an answer is not given is scored as 4. The scale contains 17 negative and 17 positive expressions about old people. The total score of the scale is calculated by adding the reversed scores of negative answers to the positive scores. The score that can be obtained from this scale developed by Kogan varies between 34 and 238. A high score from the scale points out to a positve attitude towards the elderly while a low score indicates negative attitude. In the reliability and validity studies of the scale in Turkish, internal consistency reliability coefficient (Cronbach Alpha) was found as .89 by Küçükgüçlü et al. (2011) and as .84 by Erdemir et al. (2011). In this study, internal consistency reliability coefficient was determined to be .86.

Data Analysis

Data were analyzed using SPSS software (version 12.0). The general characteristics of the participants were analyzed using descriptive statistics. In this study, variance, significance of difference between two averages test and Kruskall Wallis tests were employed according to the data characteristics.

Results

Age average of students, more than half of whom were female students (79 %) was 21,47

and their ages varied between 18 and 27 (Table 1). Other demographic variables are also included in the Table 1. When score averages of students obtained from Kogan'a scale were examined, positive score average was found as 61,22 ± 14,79 and negative score average was found as 79,98 ± 13,62 while the overall score average was found as 154,73 ± 22,08 (Table 2). When the scores that students obtained from Kogan's scale in terms of their sociodemographic characteristics, while statistically significant differences were found by the academic year (class), status of living with the elderly and status of wanting to work with the elderly, significant differences could not be found in terms of the other variables. (Table 3). Averages of scores obtained by the student from Kogan's scale increase in direct proportion to the class of the student. Score averages obtained by the first, second, third and senior students from Kogan's scale were determined as 145,31 (20,81); 152,29 (18,37); 158,38 (21,92) and 168,57 (22,57), respectively. When the relationship between age and score of Kogan was examined, a positive and significant relationship was determined (Table 4). Reasons of students for their decisions to work with old people were also included in the Table 5.

Table 1. Sociodemographic Characteristics of the Students

	X	**(SD)**
Age	21,47	1,53
	n	**%**
Sex		
Female	328	79
Male	89	21
Class		
Freshman	115	28
Sophomore	129	31
Third-Year Students	97	23
Senior	76	18
Economic status		
Good	38	9
Moderate	339	81
Poor	40	10
The longest settlement		
City	243	58
Rural Areas	77	19
Metropolis	97	23
Status of living with elderly		
Yes	231	55
No	186	45
Relationship to elderly (n=231)		
Grandfather	60	26
Grandmother	157	68
Relative	14	6
Willingness to work with elderly (n=398)		
Yes	306	73
No	92	22

Table 2. Score averages of students from KOGAN scale

	Min-max	**X**	**SS**
Negative Score	17-112	61,22	14,79
Positive Score	22-118	79,98	13,62
Total Score	94-236	154,73	22,08

Table 3. Examination of the scores obtained by the students from KOGAN scale in terms of sociodemographic characteristics

	Negative X (SD)	Positive X (SD)	Total score X (SD)
Sex			
Female	61,02 (14,81)	80,01 (13,31)	154,92 (22,28)
Male	61,96 (14,79)	80,01 (14,89)	154,06 (21,62)
	t=.531, p=.596	t=.001, p=.999	t=.327, p=.743
Class			
Freshman	66,86 (15,81)	76,38 (15,08)	145,31 (20,81)
Sophomore	62,80 (12,98)	79,09 (12,32)	152,29 (18,37)
Third-Year Students	58,96 (14,35)	81,34 (12,27)	158,38 (21,92)
Senior	52,87 (12,34)	85,41 (13,49)	168,57 (22,57)
	F= 16.566, p=.000*	**F=7.455, p=.000***	**F=20.677, p=.000***
Ekonomic status			
Good	58,11 (14,97)	80,37 (13,03)	158,26 (22,15)
Moderate	61,56 (14,44)	79,82 (13,58)	154,19 (21,90)
Poor	61,28 (17,45)	81,30 (14,94)	156,03 (24,07)
	F=.931, p=.395	F=.224, p=.799	F=.654, p=.521
The longest settlement			
City	61,15 (14,30)	79,92 (13,84)	154,77 (23,10)
Rural Areas	60,77 (15,14)	81,77 (12,23)	156,69 (19,01)
Metropolis	61,74 (15,83)	78,89 (14,18)	153,14 (21,93)
	F=.099, p=.906	F=.961, p=.383	F=.544, p=.581
Living with elderly			
Yes	60,08 (14,49)	81,17 (14,01)	157,00 (22,21)
No	62,63 (15,08)	78,58 (13,07)	151,95 (21,74)
	t=1.755, p=.080	t=1.933, p=.054	**t=2.325, p=.021***
Relationship to elderly			
Grandfather	59,80 (14,27)	80,25 (13,03)	156,45 (22,17)
Grandmother	59,45 (14,65)	81,32 (14,73)	157,73 (22,84)
Relative	68,28 (11,68)	83,57(9,58)	151,285 (14,19)
	KW=5.523, p=.063	KW=.976, p=.614	KW=.848, p=.654
Willingness to work with elderly			
Yes	59,24 (14,76)	82,10 (13,30)	158,81 (21,47)
No	67,22 (13,84)	73,36 (13,16)	142,14 (21,11)
	t=4.612, p= .000*	**t=5.538, p= .000***	**t=6.552, p= .000***

**p < .05*

Table 4. Correlation between the ages of students and their score averages from KOGAN scale

	KOGAN scale's score average	
	Correlation coefficient (r)	**p**
Age	.244	.000

Table 5. Reasons of students as regards to whether they want to work with the elderly

Students who willingness to work with the elderly	• Thinking that the elderly people need more assistance and being happy of helping them (97 expressions) • Regarding them as experienced people and wanting to benefir from these experiences (41 expressions) • Loving and getting along with the elderly as well as respecting them (67 expressions) • Thinking that they will also get old and need care (10 expressions) • Having positive experiences about the elderly in their families (8 expressions) • Thinking that they have to provide them with care as a requirement of your profession (5 expressions)
Hesitant students	• Not giving a decision yet, wanting to decide after acquiring experiences (13 expressions)
Students who do not willingness to work with elderly	• Thinking that the elderly people are obstinate • Being anxious • Lack of experience • Finding the care difficult • Personal characteristics of each individual (total 52 expressions)

Discussion

The attitude towards old people is influenced by cultural values, norms and society (Andrews, 1991). Thus, different results were obtained in the researches conducted in Eastern and Western countries with nursing students with the aim of evaluating the attitudes towards the elderly. While generally negative attitudes were reported in a study conducted in Sweden (Fageberg and Ekman, 1998; Soderhamn et al., 2001), positive attitudes were reported towards old people in a study carried out in Jordan (Hweidi and Al-Obeisat, 2005). In a study conducted in Taiwan with nursing and medicine students, it was also determined that students displayed positive attitudes towards old people (Wang, Liao, Kao et al., 2009). Similar to the eastern countries, nursing students were determined to display positive attitudes towards the elderly. In Turkey, urbanization and industrialization have caused significant changes in the family structures in the big cities. There is a rapid shift from traditional family structure to the nuclear family structure in our country. However, although the family structure has changed in Turkey, such values as perception of the elderly and family ties were preserved (SPO, 2007). In general, old people are important, strong and wise people within the family. In a study conducted in Spain, the strength of family ties in the Mediterranean culture is emphasized (Zunzunegui et al., 2001). Family ties are of great importance in Turkey, as well. It is not surprising that the young people growing in such a culture display positive attitudes towards old people.

It was reported in the literature that nursing education and working experiences with old people affected positively the shaping of attitudes of students towards old people (Hartley ve ark., 1995; Herdman, 2002; Hweidi ve Al-Obeisat 2005; Rogan and Wyllie, 2003; Sheffler, 1995; Shoemake and Bowman, 1998; Söderhamn ve ark., 2001). It was determined that as classes of nursing students participating in this study increased, their positive attitudes towards the elderly also increased. It is thought that this result can be attributed to the nursing education. Freshman nursing students do not have modules concerning the elderly in the curriculum and they do not have clinical experiences, either. They can establish contacts with old people only by participating in social sensitivity projects. As of the second academic year, social sensitivity levels of nursing students show increase, they take part in clinical practices, their communications with old people increase and they start receiving geriatrics modules. Their knowledge levels, elderly care experiences and elderly-related awareness continue to be on the increase in the third-year class. In the final year, our students, who are also intern nurses, acquire sufficient amounts of knowledge and experience to fulfill their responsibilities as clinic nurse. Final year students encounter with old people more frequently and they gain more experiences in working with old people. Thus, final year students who have gained positive experiences are thought to display more positive attitudes towards old people.

Students also get older in direct proportion to their classes in the university. It was reported in the

international studies that nursing students displayed more positive attitudes towards old people with the increasing age (Gomez et al., 1985; Holroyd, Dahlke, Fehr, Jung, Hunter, 2009; Hweidi and Al-Obeisat, 2005; Soderhamn et al., 2001). In this study, a positive and moderately significant relationship was also detected between age and attitude towards old people. Attitudes of students who get older towards the elderly become more positive. This result can be attributed both to the nursing education and the change observed in the viewpoints of nursing students with the increasing age.

In some studies conducted so far, it was determined that gender affected the attitudes towards the elderly. While in Eastern countries, male students displayed more positive attitudes towards the elderly than the female students (Hweidi and Al-Obeisat, 2005), it was determined that male students had more negative attitudes in the Scandinavian countries when compared to female students (Soderhamn et al., 2001). However, in some other studies, it was reported that gender did not influence attitudes of students towards olde people (McCracken et al., 1995; Sheffler, 1998). Our study also revealed that gender was not influential on the attitudes of students. It is thought that all male and female students had similar experiences towards old people in the Turkish society and therefore, attitudes of all students showed similarity irrespective of their genders.

More than half of the students stated that they lived with an old relative such as grandmother or grandfather in the same house. Students display more positive attitudes when they live with an old relative in the same house. In the study conducted by McCracken et al. (1995), it was determined that students having any experience of living with an old person displayed more positive attitudes. Evers, Ploeg, Kaasalainen (2011) and Holroyd, Dahlke, Fehr, Jung and Hunter (2009) also reported that spending time with an old person ensured students to have more positive attitudes towards the elderly.

There is no difference between attitudes of students participating in this study towards the elderly in terms of their economical situations. Attitudes of students towards the elderly do not vary by the economical situation. Students of the school of nursing had lived in different regions and settlements including cities, villages, metropolitan regions etc. It was also found out that settlements of students did not affect their attitudes towards old people. This result is attributed to the fact that Turkish culture prevails in all parts of the country.

In the study, the students who wanted to work with old people were those having more positive attitudes. The students' status of wanting to work with the elderly after graduation is affected posiively as the time spent with the old person increases and they get more education (Fageberg and Ekman, 1998). In another research conducted by Wang, Liao, Kao et al., (2009) in Taiwan, it was concluded that a high ratio of nursing students as much as 75 % wanted to work with old people after graduation. In this study, it was determined that the sophomore students displayed more positive attitudes towards old people when compared to the senior grade students and this result is attributed to the fact that sophomore students take part in the studies more voluntarily and therefore, they interact with the elderly more frequently. In our study, as the classes of students increased, their percentages of wanting to work with old people also increased. A high ratio of the final year students as much as almost 76 % want to work with old people. It can be thought that this situation results from increasing ages and experiences of the final year students as well as their positive attitudes towards the elderly. On the other hand, in the study conducted by Evers, Ploeg and Kaasalainen (2011), students were reported to display positive attitudes towards old people but they did not want to work with old people as they found it a monotonous, slow and heavy task. Besides, students having positive opinions about the elderly within the family want to work with them much more than the others (McCracken et al., 1995). In this study, status of wanting to work with old people and the reasons of students were examined and students stating their desire to work with old people generally pointed out their positive experiences within the family. These experiences are listed as feeling happy for helping an old person, thinking that old people are wise and experienced, anticipating that they will also get older and may need care at that time and feeling obliged to provide care to old people as a necessity of the profession.

Limitations

Data of this research were collected in a faculty of nursing providing a problem-based nursing education. Students receive a geriatrics module concerning the elderly in the second academic year in this faculty. Thus, it may not be possible to generalise the results. We must also learn the attitudes of students studying at the schools where a nursing education is provided but geriatrics is not included in the curriculum. In addition, this study reveals that nursing education changes attitudes of students towards old people by comparing the differences between the grades. However, it is believed that more comprehensive and detail data can be obtained if students are examined and evaluated throughout their university period composed of four years.

Conclusions and recommendations

In accordance with the results of this study, it can be concluded that as the grades of nursing students increase, their positive attitudes towards old people also increase, students display more positive behaviors in direct proportion to the increasing age and living an old relative within the same family also affects the attitudes of students positively. It was determined in this study that students displaying more positive attitudes towards old people would become more inclined to work with old people after they graduated from the faculty of nursing. In line with these results, it is recommended that concepts related to the elderliness and aging process are included in the course contents of undergraduate programmes providing nursing education, each student is certainly allowed to make an elderly follow-up in the respective field of practice and students are encouraged to participate in trainings, projects, panels, symposiums and congresses related to the elderly health to raise their awareness of elderly and aging. Besides, educators, nurses and trainers in the schools of nursing should provide consultancy to enable them to develop more positive attitudes towards old people. Weight and form of gerontological content within the nursing education in our country should be investigated and the existing knowledge should be evaluated. Accordingly, curriculum changes should be made. Additionally, inter-school reliability is suggested for the future researches. Then, it would be interesting to compare the findings of these researchs to the results obtained from other faculties or schools of nursing.

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Factors influencing Burnout syndrome phenomenon in social welfare institutions in the Republic of Slovenia

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Abstract

The contribution deals with the phenomenon of the burnout syndrome among employees of health care and social welfare institutions in a broad sense. The research was focused on the psychological stress experienced by workers of nursing homes, with special emphasis on the consequent reactions, which may already represent signs of burnout or its onset. We used two questionnaires, which were created on the basis of a questionnaire on burnout (MBI-HSS - MBI-Human Service Survey) and a customer satisfaction questionnaire (the Job Descriptive Index). As an additional research instrument, we made a list of psychosomatic disorders and a structured interview with closed questions.

Early identification of risk factors that increase susceptibility to the burnout syndrome is essential for organizations in order to design effective strategies to reduce and prevent its consequences. The connection between individual factors and the experience of exhaustion was calculated by linear regression. The calculated linear regression model significantly predicted exhaustion. The experience of exhaustion was negatively related to job satisfaction, and the number of psychosomatic factors was positively connected to job satisfaction. Work satisfaction is negatively correlated with experiencing burnout, the correlation between work satisfaction and the number of psychosomatic factors is positive. Other correlations close to statistical significance are between work satisfaction and feeding, number of children and work difficulty.

Key words: Burnout syndrome, social care institutions, health care, work satisfaction.

Introduction

With the changes in working conditions, the ageing of the population and the processes of transition and globalisation, the amount of psychological stress present in our environment has risen considerably. A large part of it can be attributed to changes in the labour market which have produced an increase in the share of employees working in the service sector. The service sector by itself is characterised by generating more psychological stress on the workers than other sectors (Rozman, 2010).

The efficiency of managing psychological stress depends heavily on the working environment. An analysis of the psychosocial risk factors in the workplaces of small and medium-sized companies has shown that the origins of stress are often specific and depend heavily on the actual working conditions as well as on the fitness of an individual to handle the changing conditions and new demands (Blažič, 2011).

The results of the research on the burnout syndrome in Slovenia carried out by the Institute for Human Resources Development (Inštitut za razvoj človeških virov) are worrying: only 40 % of the respondents did not show any signs of burnout, 30 % showed signs of exhaustion (first stage of burnout), 22 % experienced a feeling of entrapment (second stage) and 8 % were burned out (half of which had already had a nervous or adrenal breakdown). At the same time it was shown that a large number of people evaluate themselves based on how high their productivity is and that living conditions in Slovenia are very stressful (Pšeničny, 2008).

Health care services face many social, psychological and medical challenges. This means that members of nursing teams often have to deal with events that are very stressful. Tasks that are unpleasant and fear-inducing are therefore inherent parts of health care services. Medical workers often have to witness pain, suffering and death (Kaučič, 2002). Although performing health care can be a rewarding task, exhaustion and emotional stress are common reactions to it. The disturbances can

be expressed in the form of physical symptoms, such as peptic ulcers, high blood pressure and sleep disorders (Kaučič, 2002). The nursing staff regard their positions as being »sandwiched in« between the needs of the patients and the demands of the medical team (Babič et al., 2007). Patients expect help and compassion, while doctors delegate tasks and responsibilities. The medical nursing staff are faced by numerous situations of distress which are permanently absorbed.

Maslach (1993) pointed out a problem which was revealed in burnout studies, i.e. the shift of focus from the influence of social relationships to the influence of working conditions, particularly the aspects of industrial and organisational psychology. Despite many potentially stressful tasks that performing health care demands, lately researches have been focusing on the aspects of supervision, ergonomics and rewarding and supporting the nursing staff (Maslach & Leiter, 2002). Mihalič (2008) highlights that job satisfaction is a distinctly positive emotional state of an individual and is dependent on the manner in which one perceives and evaluates their work, working environment, work experiences and on the general disposition towards all the elements of work and working environment, the sum of it all representing an individual‘s effective reaction to work, working environment and working conditions.

According to Rakovec-Felser (2006), when we consider the emotional capacity of the medical workers (mainly the ability to feel empathy) the following questions are important: how much of this capacity an individual brings into their working environment and how much of it is reduced by the nature of their work, poor organisation, work climate and interpersonal relationships.

The roles that an individual is assigned at his workplace demand specific behaviour, while the organisational processes define the time and place where his or her tasks will be performed. If we assume that an individual is capable of applying the techniques and skills he or she learned in practice, there remains a question of whether these techniques are connected to the burnout syndrome. With this in mind, the goals of the research were:

- Identify the effects of the burnout syndrome on medical workers who provide direct health care to the older population.
- Obtain information on the phenomena of psychosomatic symptoms: insomnia, headaches, neurosis, backaches, anxiety, undernourishment, overnourishment, frequent infections etc.
- Identify the cause of mental stress and mental disorders in employees of homes for elderly persons.
- Highlight the risk factors which play an important role in burnout experience.

Research methodology

Research questions

In the study we were aiming at answering the following questions related to exhaustion:

- Does the experience of exhaustion vary significantly in relation to sex, age and basic occupation of nursing staff, working conditions, work situation and time of working directly with the elderly?
- Is the experience of exhaustion negatively related to job satisfaciton?
- Is the experience of exhaustion positively related to psychosomatic symptoms?

Research methods and research design

We used two questionnaires which were formed on the basis of the MBI-Human Service Survey (MBI-HSS) and the Job Descriptive Index (JSI). As an additional research instrument we prepared a list of psychosomatic disorders and a structured interview with closed-ended questions. The list of psychosomatic disorders was made on the basis of international classification of psychosomatic disorders and disesases (ICD-10).

Sampling and research execution

In the period lasting from November 2009 until January 2010, 500 questionnaires were sent and 228 structured interviews were carried out in ten randomly selected elderly homes: Ljubljana Centre, Tabor-Poljane, Ljubljana Šiška, Ljubljana Bežigrad, Kočevje, Sežana, Črnomelj, Domžale, Litija, Dom obn Savinji Celje and Koper.

The reliability of the sample was calculated on the basis of Cronbach‘s alpha value for three dimensions: experience of burnout (a = 0.78), job satisfaction (a = 0.71) and difficulty of work (a = 0.77). The

Cronbach's alpha value exceeded 0,70 in all three measured dimensions. The treshold of 0.70 is the value above which samples are considered to be reliable in literature (Nunnally and Berstein, 1997).

For the sake of easier analysis of the hazards medical workers are facing, all of the job positions (graduated nurse, nurse, hospital attendant, attendant, cleaner, physiotherapist, assistant director of health care, work instructor, social worker and so on) were grouped into the following categories: nurses, graduated nurses, hospital attendants and others.

Statistical method

The respondents were measured and connected to the dependent variable »experience of exhaustion«. A total of 55 independent variables were measured. Each variable contained a subvariable, meaning a total of 220 items were studied. Experience of exhaustion was measured with 31 factors which we combined into a derived variable using the Teeri et al. (2008) method. The measuring scale used was: 0 = No, ½ = Sometimes, 1 = Yes. The experience of exhaustion was connected to the independent variables in the subsequent statistical analysis of the results.

For identification of the attributes of job satisfaction, 23 factors were included and measured. Once again, the measuring scale was: 0 = No, ½ = Sometimes, 1 = Yes. To measure the difficulty of work, we formed a derived variable »work difficulty« which includes five work tasks. The measuring scale was: 1 = work task is performed, 0 = work task is not performed.

Additionally we measured the presence of psychosomatic symptoms whose occurences we connected to sociodemographic data of the respondents (age, sex, marital status, number of children, level of education, occupation, number of work shifts, coworkers on the same shift, years of employment on the current position, type of employment contract).

The connection between individual factors and the experience of burnout was calculated with linear regression. The value of beta coefficient, t-value and p-value were determined. The statistical analysis was done with SPSS 15.0 software (SPSS Inc., Chicago, IL). The p-value of < 0.05 indicated statistical significance.

Results

Responsiveness and structure of the respondents

Out of 500 questionnaires sent, 228 were adequate for further analysis, or an average of 22 questionaires and 22 interviews per each institution. The response rate for both instruments was 45.6 %. The survey included 215 females (94.3 %) and 13 males (5.7 %). The average age of the respondents was 42.1±8.9 years. The dsitribution of education was as follows: elementary school – attendants, cleaners (N=29), specialized school – hospital attendants (N=50), high school – nurses, hospital attendants, attendants, work instructors (N=96), college, faculty, postgraduate education – assistant directors, graduated nurses, physiotherapists, social workers (N=49). Concerning marital status, 31 respondents were married (53.9 %), 48 lived in a civil union (19.8 %), 35 were single (14.4 %), 22 were divorced (9.1 %) and 7 were widowed (2.9 %). The most common number of children was 2 and the least common number of children was 4. 24.3 % of the respondents had been working in this field for 10 – 20 years, 21 % for 21 – 25 years, 10.4 % for 26 – 30 years and 4.2 % for 31 – 35 years.

Results of data analysis

By organising and analysing the obtained empirical data, insight was achieved into the factors that influence the occurence of the burnout syndrome. They were classified into categories and are presented below.

Psychosomatic symptoms and diseases

A psychosomatic list was created to measure 11 psychosomatic symptoms and diseases: respiratory diseases, cardiovascular diseases, eating disorders, digestive diseases, endocrine and metabolic diseases, alergies, skin diseases, headaches, insomnia, gynecological diseases, myopathies and connective tissues and joint diseases. These psychosomatic symptoms were added up to get the following picture: out of 228 respondents, 46 (20.2 %) showed no psychosomatic symptoms that would reduce their ability to perform work tasks or force them to take sick leave. The remaining 182 (79.8 %) respondents exhibited or had exhibited in the past an average of 2.3±2.0 different psychosomatic symptoms, which means that on average

more than two psychosomatic symptoms were identified per person.

The respondents particularly pointed out back pains (17.4 %), followed by locomotor system disorders (9.9 %), respiratory diseases (6.7 %), cardiovascular diseases (6.1 %), digestive diseases (2.4 %), urinary tract diseases (1.6 %) and skin diseases (2.0 %). 7.1 % of respondents have had an operation. 1 % of respondents selected the **»other** problems and diseases« category. 31.7 % of respondents claim they suffer from a chronic disease.

Job satisfaction

Job satisfaction was determined by way of interview which comprised questions bearing on the work tasks and work situations, interpersonal relationships between caregivers and care-recievers and employees and their relatives. The way in which respondents experience old age as a life period was also examined.

For the purpose of this research we used the definition of Nolan, Cushway et al. (1995), who defined job satisfaction and job dissatisfaction as the result of indviduals‘ dispositions towards work, work-related factors and life in general. In this case, disposition towards work is the emotions the worker experiences while working and his readiness to react in appropriate ways to different work-related factors.

The first set of questions focused on the way medical workers experience different attributes of their work. We were particularly interested in whether they are under pressure and whether they make mistakes due to the short amount of time available for the treatment of each care-reciever (24 minutes per care-reciever). 95 respondents (36 %) said they are afraid to make a mistake, 70 (30.5 %) are occasionally afraid and 63 (27.6 %) are not afraid. Considering these numbers, we can conclude that the pressure produced by short periods of time in which tasks must be done is an additional risk factor contributing to the burnout phenomena. The fear of making a mistake can therefore be a factor in support of a burnout experience. The respondents which said they were afraid are under greater pressure that those who said they were not afraid.

Unphysiological working conditions arise in cases where work and working time are poorly organised. The rhytm and intensity of work can produce physical and psychological pressures and put strain on indvidual organs and organ systems (Stričevič et al. 2009). The feeling of fear can be intensified by certain personality traits and factors arising from the work situation (supervisors‘ high expectations, unrealistic demands by the elderly and their families, poor social security of the occupation etc.). The presence of fear can be interpreted as a factor of work with the elderly, who need more attentive and careful treatment. The result of this is that the majority of employees feel that their work is psychologically stressful and consequently the source of numerous stressful situations. 195 (75.9 %) respondents is of the opinion that their work is not appreciated enough, which most likely means that they receive signals from their social environments telling them that their occupation is not as respected as they think it ought to be. We see this as a positive note, in the sense that the respondents apparently respect their work and profession despite the lack of attention and appreciation from the society. On the other hand, the society‘s opinion can make it harder for individuals to identify positively with their occupation and can lead to internal struggles within these indviduals.

36.4 % of respondents do not think their job is dirty. They think it is worth the effort and 76.3 % of them say that they are very engaged in their work and that it matters to them how well they perform their work tasks. All of this shows that tehe respondents are taking their occcupations very seriously. Only 1.2 % thought that they were lazy workers. We did not verify whether these responses indicated the presence of certain personality traits or merely lack of motivation. The latter might be a consequence of different factors, which nonetheless must be similar to the factors which produce exhaustion.

We expected the dispositions of interviewees to vary according to how they experience old age as a life period. Almost half of them (49.1 %) thought that old age is a difficult life period. 70.6 % thought that old age is a period of exhaustion, while 21.9 % answered that old age is beautiful. Another thing revealed was the connection between how they experienced exhaustion and whether they felt terrible about the fact that they will be old one day. The interviewees who did not have pessimistic views towards old age were less li-

kely to experience burnout or experienced it in a less intense manner. Similarly, differences in the experience of burnout were revealed between those who thought old age can be a quality life period and those who thought otherwise. The former experienced burnout less often and less intensely than the latter. These differences are not to be explained simplistically. Meaning, it is not easy to distinguish between the consequence and the cause. It is entirely possible that negative disposition towards old age (and consequently towards working environment) can be one of the many factors contributing to the lack of motivation and the onset of exhaustion. It makes it harder to attribute meaning to one's work and therefore harder to get a sense of accomplishment and enjoyment out of it. However, the opposite could also be true. An employee who is overburdened and receives negative experiences from working with difficult individuals can start changing his or her views towards old age, becoming more pessimistic which adds another risk factor for the phenomenon of burnout.

The interviewees pointed out that the most difficult task is nursing, with 45.8 % replying that they are under extreme stress when handling immobile elderly people. In addition, 23.7 % said that they have difficulties carrying out medical interventions. A lot of medical procedures and interventions is due to the changed health structure of the elder population. According to data provided by social welfare institutions, 85 % of elderly people in elderly homes can be classified as partially or completely dependent on help from members of medical and nursing teams (SSZS, 2011).

Feeding the elderly people is another task which takes lot of time every day, due to possible health complications in connection to food swallowing (danger of asphyxiation if feeding is too quick). According to professional reccomendations, it takes an average of 20 minutes to feed an elderly person who has difficulties swallowing. A nurse working in the hospital department of an elderly home must feed 7 to 8 elderly persons per day, on average.

The respondents also pointed out the process of preparing and handing out drugs. It is a time-consuming process which needs to be carried out carefuly, due to the fact that each elderly persons receives on average 6 to 13 drugs at one time. Another difficult task according to the respondents is helping the elderly with everyday services, while 6 % also feel burdened by work organisation and 7 % by work supervision.

Only 7.5 % answered that they are experiencing difficulties while dealing with the elderly persons. We assume that this is the portion of population who finds working with the elderly unsatisfying and hence also tires more quickly.

Results of sociodemographic characteristics of respondents

Less correlations between burnout and characteristics of the respondents were found than anticipated. The most prominent one was the correlation with job satisfaction ($p = 0.004$), followed by the correlations with psychosomatic disorders ($p = 0.038$), work difficulty ($p = 0.075$) and food distribution and feeding ($p = 0.084$). There is a hint to the correlation between the number of children and burnout ($p = 124$), but it is not statistically significant. There is no statistical connection between burnout and years of service, age, sex, marital status, level of education or type of employment relationship.

Results of independent factors in correlation with the experience of burnout

he research was concluded by identifying the correlation and the share of beta coefficient variance between risk factors and the experience of burnout. The value of R (multiple correlational coefficient of the linear regression model) is 0.579, R^2 is the share of variance for burnout experience and its value is 0.335. It is clear from these results that job satisfaction is negatively connected to burnout experience (beta coefficient = -0.23), while the connection between the number of psychosomatic factors and burnout experience is positive (beta coefficient = 0.17). The share of coefficient for the food distribution and feeding variable is also close to being statistically significant (beta coefficient = 0.15), followed by the number of children (beta coefficient = -0.12) and work difficulty (beta coefficient = 0.15).

All this shows that employees are exposed to burnout experiences which tells us that most of them have a predisposition for the burnout phenomenon.

Discussion

A report by the International Council of Nursing (INC) (2007, 2010) claims that the gap between what nurses are able to accomplish and what their occupation demands of them is endangering their health as well as their patients‘ health. ICN clearly highlights the fact that a safe working environment is the basis for the positive outcome of the healing process of the patient. Working in the field of medical care is one of the most stressful professions. Morton-Cooper (1984) points out that life expectancy for nurses is only one year higher than that for miners. Burnouts occuring in medical care staff are worrying from several perspectives. A burned-out individual‘s physical and mental health is affected, which leads to reduced capacity for work. Consequently, the employing institution suffers financial losses because of sick leaves. The respondents claimed they were physically and psychologically burdened by working with the elderly. They listed several particularly difficult tasks: nursing, drug preparation and distribution, feeding elder persons with medical complications, and pointed out unbalanced relationships between relatives and employees, lack of appreciation for their work in the society, lack of staff and work in shifts. Uneven distribution of work and short amount of time given to complete each work task are sources of stress which leads to elevated blood pressure, which may result in cardiovascular diseases (Milutinović, 2009). The inevitable occurrences of death are an additional factor which results in workers‘ exhaustion and psychosomatic disorders. The incidence of psychosomatic disorders serves as a good illustration of the actual state of health of the employees of social welfare institutions. On average each respondent exhibited 2 psychosomatic disorders and respondents (88 %) sought medical help in the last 12 months due to psychosomatic-related problems. 44 % of them experienced panic attacks and depressions, 28 % experienced stress, 16 % had mental problems and only 1.2 % stated other problems, which shows how urgent it is to find a solution to the problem of burnout.

The research focused exclusively on Slovenian homes for elderly people. After studying other researchers‘ work we could tentatively compare our study to the research done by Zeller, Hahn, Needham, Kok, Dassen and Halfens (2009) who found out that the most significant source of stress for the employees of elderly homes and visiting nurses is verbal violence from patients (threats, insults). Selič (2010) points out in her study that employees in primary medical care are burdened and Kržišnik and Čuk (2010) warn that the workers in psychiatric medical care experience stress while working with geriatric patients.

The implication is that hospitals, nursing homes and homes for the elderly around the world experience similar problems to those in Slovenia, i.e. lack of staff, low levels of education and work-related stress (physical and psychological) which all contribute to burnout phenomena. Our research was limited to ten homes but it could nonetheless be concluded that the problem of burnouts is indeed present in social welfare institutions and needs to be examined and studied seriously.

Judging from the obtained results, it is urgent that the risk factors contributing to the burnout syndrome are recognised in time. Therefore social welfare institutions need to start developing a procces of evaluation of psychosocial and medical risk factors contributing to work stress, or face large financial losses due to stress-related sick leaves. On the other hand, institutions should provide moderate work demands, give their employees a chance to choose and control the work tasks they perform, reward them for good performance, strengthen coworker relationships, promote honesty, respectfulness and fairness, and most importantly, help employees to feel that what they do is meaningful and appreciated.

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Effect of music on the patients anxiety that endoscopy will be applied

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Abstract

Endoscopic interventions are very difficult, stressful invasive diagnostic and treatment models for patients that are caused anxiety. In order to reduce anxiety some complementary methods are applied such as therapeutic communication, music therapy. This study was determined to examine that the effect of music on the patients anxiety that lower and upper gastrointestinal system (GIS) endoscopy will be applied. The studying group was constituted total 72 patients that 36 of experimental group other 36 of control group. The questionnaire form which was prepared by researchers including socio-demographic variables and the State Trait Inventory which was used to applied patients before and after music therapy.According to the study, it was found statistically significant difference between the experimental group' pre-test and post-test state anxiety scores ($p<0.05$). And also statistically significant difference was found between experimental groups and control groups of post-test state enxiety scores ($p>0.05$). As a result, music reduced to patients' anxiety level that gastrointestinal endoscopic intervention will be applied. Music to decrease anxiety can be suggested to use as a independent nursing initiative.

Key words: Endoscopic intervention, music therapy, anxiety.

Introduction

Endoscopic interventions are very difficult, stressful invasive diagnostic and treatment models for patients(Hassan-El et al.,2009; İşler et al.,2001). Anxiety is occured many reasons such as strangeness of hospital environment, necessity to communication with strangers, doctor or nurse to use of medical terms, the use of unknown medical instruments, diagnostic and therapeutic procedures to applied that patients who have some health problems to go to health facilities (Ekiz & Göz 2005; Güneş 2001; Pinar&Yürügen1994). Anxiety is the feeling of uneasiness and fear in the long-term concern that perceived as a threat to the life or disturbing one or many intensity of the situation. Level of anxiety (long term or intensity) hurts people, reduces the quality of life, destroys social life and causes the loss of labor force(Güneş 2001). Therefore, during recent years, patients are evaluated in a holistic approach that aims to decrease anxiety, to increase satisfaction and improve quality of life during invasive procedures such as endoscopy.

Studies that have done for this purpose focus on the complementary methods such as therapeutic communication, fantasies methods, aromatic methods, therapeutic touch, music, drawing attention to another direction, hypnosis and acupuncture (Arslan et al.,2008; Thorgaard et al., 2004;Yung et al.,2002; Tonnesen et al.,1999; Heitz et al.,1992). Music is an art that expresses feelings and thoughts by sounds or expresses in tones of order within esthetic. Recognitioned that music is not only a fun tool and also music reflects the human psyche, emotion and thought world of human has enabled to make many scientific researchs of music on people(Ak 1994). Music therapy is defined as form of controlled listening of music that affects patients' physiological and psychological during treatment(Chlan & Tracy 1999). Lower voice pitch and lower tempo music reduces the ability of neuronal migration, arranges an uncomfortable feelings, and also affects the brain's limbic system which is the center of emotion and excitement. Music acts important role that creates physiological and psychological changes on the neuroendocrine system and autonomic nervous system. By activating the parasympathetic nervous system that causes a reduce in physiological signs such as blood pressure, pulse and respiratory (Arslan et al.,2008; McCaffrey & Locsin 2002;Güngör 1999). After reviewing the literature, research de-

termined that practice of using music is limited on the endoscopic interventions (Uçan et al.,2007) although different research groups examined the effect of music on anxiety (Arslan et al.,2008; İşler et al., 2001; Güngör 1999). This study was determined to examine that the impact of music on the patients anxiety that lower and upper gastrointestinal system (GIS) endoscopy will be applied.

Materials and methods

Design

Research was performed as a randomized controlled and experimental work, and patients who have applied to hospital for have made to gastrointestinal endoscopy to university hospital which department of Gastroenterology Endoscopy Unit were taken to study. The research was comprised patients who were willing to participate in research, good communication, hearing, non-speech problem, prior to any drug that is not an analgesic and anesthic, which is over 18 years of age and who have never made endoscopy of 36 experimental and 36 controlled that 72 patients were taken to the study.

The study was single-blinded. Patients were agree to participate in the study were randomized to either a music group or a non music group (usual care). The randomization list was prepared by EN. EN had no contact with study participants. Randomization was implemented by SA. Randomization was carried out by the use of opaque envelopes, half of which containing a piece of paper that said "music," and the other half containing a piece of paper that said "no music."

Data collection

Data were collected through questionnaire including items related to the socio-demographic variables, which prepared by researcher and State Trait Anxiety Inventory (STAI I,STAI II) was used to evaluate for assess level of anxiety before and and after treatment. This scale was developed by Spielberger and his colleagues in 1970 that can be applied above the age of 14(Spielberger et al.,1970). Its Turkish adaptation, validity and reliability were made by Öner and Le Compte (Öner & Le Compte 1983). State anxiety scale determines that at a certain moment and under certain conditions how he felt himself on the other hand trait anxiety scale determines that the conditions of the individual how he felt himself as an independent. Test is filled by self. This test is consisted of 20 questions to measure state anxiety and the ongoing anxiety. State Anxiety Scale is a very sensitive tool to assess that indicating a sudden change in excitemental reactions. The Trait Anxiety Scale which consists of 20 items in the second part of the inventory intends to measure of person' anxiety continuity in general of life. Scores are between 20(lower anxiety) and 80(higher anxiety). 36 and lower point signs that there is not anxiety, 37- 42 point signs medium level anxiety, 43 and higher point signs that high anxiety. In general, if state and trait anxiety point is high indicated that anxiety level is high. If scores are above 60, Professional help is required. Necessary permissions were obtained from endoscopy unit, university ethics committee and patients who have applied to make endoscopy before the study by the institution. Music preferences were asked to patients who will be treated. State Trait Anxiety Inventory was applied to patients before intervention and music which chosen by patients was listened with the headphones to patients for 15 minutes in the waiting room. State anxiety scale was applied to patients after the treatment.

Statistical analysis

The data was evaluated by the SPSS 11.0 packet program. Descriptive tests, the chi-square test were used in the statistical analysis of data. T- test was used for independent groups. Significance level was taken as $p< 0.05$.

Results

Descriptive properties of the experimental and control group of patients are given in table 1. Statistically significant difference was found between the groups of descriptive properties.

There was not found any statistically significant difference between experimental groups and control groups of trait anxiety average score($p>0.05$). It was found statistically significant difference between pre-test and post-test state anxiety average scores of the experimental group' ($p<0.05$). And also statistically significant difference was found pre-test and post-test state anxiety average scores of control group ($p<0.05$) (Table 2).

Table 1. Characteristics of the Sample

Variable	Music (S=36)		Control(S=36)		P Value
	Number	%	Number	%	
Age	X ± SD=43.55±11.93		X ± SD=42.22±13.71		X^2= 580.00 Df=1 P= 0.359
Gender Female Male	 21 15	 58.3 41.7	 22 14	 61.1 38.9	X^2= 2.258 Df=1 P= 0.133
Marital status Married Single	 30 6	 83.3 16.7	 29 7	 80.6 19.4	X^2= 0.887 Df=1 P= 0.346
Education Illiterate Literate Primary school Secondary school University	 1 - 12 14 9	 2.8 - 33.3 38.9 25.0	 2 1 9 14 10	 5.6 2.8 25.0 38.9 27.8	X^2= 10.563 Df=12 P= 0.610
Working status Yes No	 22 14	 61.1 38.9	 20 16	 55.6 44.4	X^2= 0.286 Df=1 P= 0.593
Place of residence City Town	 32 4	 88.9 11.1	 33 3	 91.7 8.4	X^2= 1.636 Df=1 P= 0.201
Income level Income > expenditure Income = expenditure Income < expenditure	 6 16 14	 16.7 44.4 38.9	 9 15 12	 25.0 41.7 33.3	X^2= 3.633 Df=4 P= 0.458
Hospital experience Yes No	 26 10	 72.2 27.8	 29 7	 80.6 19.4	X^2= 3.735 Df=1 P= 0.06
Procedure type Endoscopy Colonoscopy	 21 15	 58.3 41.7	 21 15	 58.3 41.7	X^2= 0.735 Df=1 P= 0.391
Information status Yes No	 15 21	 41.7 58.3	 13 23	 36.1 63.9	X^2= 0.994 Df=1 P= 0.319

Table 2. Comparison of state anxiety scores within music and control groups

Groups	Measures				
	Trait anxiety Pre-test Mean ± SD	signifance level	State anxiety Pre-test Mean ± SD	State anxiety Post-test Mean ± SD	Signifance
Music	44.47 ± 9.96	t=0.766 p= 0.443	48.80 ± 13.11	41.13±12.65	t=4.678 **p= 0.000**
Control	42.63 ±8.68		43.52 ±12.17	47.62±13.64	t=3.522 **p= 0.001**

Table 3. Comparison of state anxiety scores between music and control groups

Measures	Groups	Number	Mean ± SD	signifance level
Pre-test	Music Control	36 36	48.80 ± 13.11 43.52 ±12.17	t=1.673 p= 0.103
Post-test	Music Control	36 36	41.13±12.65 47.62±13.64	t=2.097 **p= 0.04**

When experimental and control groups pre-test and post-test state anxiety average score were examined, a statistically significant difference was not detected that experimental and control groups' pre-test state anxiety average score($p>0.05$), but statistically significant difference was found between experimental groups and control groups of post-test state anxiety average scores ($p<0.05$).

Discussion

The most striking result of the study of music that has used as a nursing initiative reduced anxiety of patients that gastrointestinal endoscopy will be applied. Music which has chosen by patients was listened with the MP4 to patients for 15 minutes. Results shows that music is a cheap, reliable and simple way to reduce anxiety of patients that gastrointestinal endoscopy will be applied. In conclusion, there was not found any statistically difference between experimental groups and control groups of trait anxiety average score. Groups to be similar is desirable result. It was found statistically significant difference between the experimental group' pre-test and post-test state anxiety scores ($p<0.05$), and also statistically significant difference was found between experimental groups and control groups of post-test state anxiety average scores ($p<0.05$). El- Hassan and his collegues(2009) result of study on the impact of music on the anxiety patients that applied for endoscopy found that there was a statistically significant difference between pre-test and post-test anxiety average scores of the experimental group'.Hayes et al.(2003) were detected that "music reduces anxiety significantly before gastrointestinal intervention" in their randomized controlled study which is called as a music initiative to reduce anxiety before gastrointestinal intervention. Chlan and his collegues(2000) reseach which is called the impact of music on satisfaction, anxiety, discomfort of the patients that sigmoidoskopi will be applied has determined that music group' anxiety level was lower. Palakanis and his collegues(1994) found study on the sigmoidoscopy that groups which were listened music anxiety level lower than standart care area. Bechtold and his collegues(2006) study on the patients detected that patients' who were listened music anxiety level was lower than patients who were not listened music. Rudin and his collegues'(2007) in randomized controlled meta-analyse study which is called "music during the endoscopy" indicated to confirm Lopez-Cepero and his collegues(2004), Hayes and his collegues(2003), Chlan and his collegues (2000) studies that is listened to music reduces anxiety level of patients during the endoscopy. Smolen and his collegues(2002) detected that statistically significant difference was not found between the experimental group' and control groups' pre-test and post-test anxiety average scores however they assigned in the experimental groups' anxiety average scores'was falled.

As a result, music reduces patients' anxiety level that gastrointestinal endoscopic intervention will be applied. Music to reduce anxiety can be suggested to use as a independent nursing initiative. This study can be shed light to other researches which is planned for other patients groups.

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Congenital anomalies of the coronary artery origin: Diagnosis with multidetector CT angiography

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Abstract

Objective: To review the multidetector computed tomography angiography (MDCTA) features of the congenital anomalies of coronary artery origin and to determine their prevalence in 1500 patients.

Methods: The 16-slice coronary MDCTA of 1500 patients performed in a single center between the period of June 2005 and December 2011 were retrospectively reviewed.

Results: The prevalence of the anomalous anatomical origin of the coronary arteries was 1.87% (n=28). The hemodynamically significant anomalies were observed in 9 patients (0.6%). Five cases of anomalous left main coronary artery (LMCA) originating from the right coronary sinus were detected (0.33%). One case of anomalous left coronary artery arising from the pulmonary artery (ALCAPA) was seen (0.07%). One case of single coronary artery originating from the right coronary sinus was observed (0.07%). One case of single coronary artery arising from the left coronary sinus with absent right coronary artery (RCA) was detected (0.07%). Eight cases of anomalous left circumflex artery (CX) originating from the right coronary sinus were seen (0.53%). Eight cases of anomalous RCA arising from the left coronary sinus were observed (0.53%).

Conclusion: The MDCTA allows an exact and noninvasive definition of the origin and course of the anomalous coronary arteries.

Key words: Coronary arteries, anomalous origin, multidetector computed tomography, angiography.

Introduction

The congenital anomalies of the coronary arteries are rare. They have an incidence of 1-2% in the general population [1]. Most of these anomalies are generally asymptomatic and detected incidentally. In some cases they may cause life-threatening symptoms such as myocardial infarction, arrhythmia and sudden death especially among young athletes. For several decades, the diagnosis of the coronary artery anomalies has been made by the conventional coronary angiography (CCA), which is an invasive technique. The CCA is based on a limited number of imaging planes and offers only a two-dimensional (2D) visualization of the coronary anatomy as oppose to the cardiac computed tomography (CT). The conventional cardiac CT applications were restricted to the cardiac chambers, myocardium and coronary calcifications. The advent of the single-slice helical CT in 1996 was not able to supply imaging of the coronary arteries with no motion artifact. Within the past decade, significant developments have been made in cardiac imaging. Recent modalities consist of coronary magnetic resonance angiography (MRA), electron beam computed tomography (EBCT) and coronary multidetector computed tomography angiography (MDCTA). The development of multidetector computed tomography (MDCT) and electrocardiographically (ECG) synchronized scanning enabled appropriate CT imaging of the coronary arteries. The slice thickness has diminished from 3-5 mm to 0.6-0.7 mm. The scanning time has decreased from 40-50 s to 6-7 s during a single breath hold. The advent of various post-processing techniques including maximum intensity projection (MIP), multiplanar reformation (MPR), curved multiplanar reformation (CMPR) and volume-rendering technique (VRT) has allowed three-dimensional (3D) imaging of the coronary arteries. Due to the dramatic improvement of the MDCT technology in the past decade, the coronary MDCTA has become a

more and more important modality in the diagnosis of the coronary artery diseases. The ECG-gated MDCTA is a noninvasive imaging technique, which has perfect spatial and temporal resolution for detecting the origin and the course of the anomalous coronary arteries.

The main coronary arteries consist of the left main coronary artery (LMCA), the left anterior descending artery (LAD), the left circumflex artery (CX) and the right coronary artery (RCA) (Fig.1). The LMCA originates from the left posterior coronary sinus. The length of the LMCA ranges from 0.5 to 2 cm. The LMCA bifurcates into the LAD and CX. Sometimes the LMCA releases the ramus intermedius branch, which has a course similar to the first diagonal branch of the LAD. The LAD courses in the anterior interventricular groove towards the apex. It supplies the anterior free wall of the left ventricle with diagonal branches and the anterior interventricular septum with septal branches. The CX courses in the left atrioventricular (AV) groove and gives rise to the obtuse marginal branches supplying the lateral wall of the left ventricle. The RCA originates from the anterior right coronary sinus below the origin of the LMCA. The RCA courses in the right AV groove towards the posterior interventricular septum. Generally, the conus artery is the first and the sinoatrial node artery is the second branch of the RCA. The acute marginal branch originates from the junction of the middle and distal segments of the RCA. The distal RCA bifurcates into the posterior descending artery (PDA) and posterior left ventricular branches in the right dominant system. The artery that courses in the posterior interventricular groove and releases the PDA is regarded as the dominant coronary artery. Approximately 85% of the individuals have right dominance. Left dominance, in which the PDA and the posterior left ventricular branches originate from the CX is detected in 7-8% of the individuals. In codominant system, which has a range of 7-8%, the PDA originates from the RCA and the posterior left ventricular branches originate from the CX [2-6].

The classification of the coronary artery anomalies is defined according to the origin, course and termination of the coronary arteries [7] (Table 1). Coronary artery anomalies might also be categorized as hemodynamically significant or insignificant. The hemodynamically significant anomalies include anomalous origin of the LMCA or RCA from the pulmonary artery, anomalous origin of the LMCA or RCA from the opposite coronary sinus with an interarterial (malign) course between the aorta and pulmonary artery, myocardial bridging and congenital coronary artery fistula. Hemodynamically significant anomalies may lead to perfusion anomalies, myocardial ischemia, arrhythmia or sudden death. Surgical treatment is generally suggested for these anomalies [5]. Our study included the congenital coronary artery anomalies of origin. The purpose of our study was to retrospectively review the 16-slice CT features of these coronary artery anomalies and to determine their prevalence in 1500 patients.

Methods

1500 patients (879 males, 621 females; age range, 16-81 years) who underwent 16-slice coronary MDCTA between the period of June 2005 and December 2011 in a single center were included in the study. Most of the patients were either asymptomatic or had high risk factors for the coronary heart disease. Some of the patients were scanned owing to the known or suspected coronary artery disease. The caffeine and nicotine were avoided for 24 hours before the procedure. The patients who had a heart rate more than 65 beats per minute were premedicated with oral administration of 50 mg propranolol two hours before the scan. An antecubital vein and a 18-20 gauge intravenous cannula were preferred for the venous access. An amount of 90-100 mL of contrast media with an iodine concentration of 370-400 mg/mL and a following 50 mL saline were administered to the patient with a flow rate of 4-5 mL/s. The MDCTA examinations were carried out by retrospective ECG-gated 16-slice CT scanner (Lightspeed 16, GE Medical Systems, Milwaukee, Wisconsin, USA). The scan area was from arcus aorta to the cardiac apex. The imaging parameters were; tube voltage of 120 kV, tube current of 400 mA, table feet of 3mm/rotation, gantry rotation time of 500 ms, slice thickness of 0.625 mm and pitch of 0.3. The scan was completed within a single breath hold of 15-20 s. The CT data were reconstructed in different cardiac phases to get images with no motion artifact. The optimally reconstructed images were transferred to the workstation AW4.2 (Advantage Win-

dows 4.2, GE, Medical Systems, Wisconsin, USA) for processing. The images were evaluated in axial plane and then with post-processing techniques such as MIP, MPR, CMPR and VRT. The MDCTA examinations were evaluated by two radiologists who were experienced in cardiovascular radiology. Determination of the origin and course of the coronary arteries was performed. The analyses consisted of the anomalous origin, retroaortic, interarterial, prepulmonic and intraseptal (subpulmonic) courses of the coronary arteries.

Results

The prevalence of the anomalous origin of the coronary arteries was determined as 1.87% (n=28) in our study (Table 2). The hemodynamically significant anomalies were observed in 9 patients (0.6%). In five cases (0.33%), an anomalous LMCA originated from the right coronary sinus. The LMCA followed a retroaortic course in three cases and an interarterial course between the aortic root and the pulmonary artery in the remaining two cases (Fig. 2). In one case (0.07%), an anomalous left coronary artery arising from the pulmonary artery (ALCAPA) was observed (Fig. 3). In one case (0.07%), a single coronary artery originating from the right coronary sinus was seen. In this case, the single coronary artery trifurcated into the LAD, CX and RCA. The LAD followed a prepulmonic, the CX followed a retroaortic and the RCA followed a normal course (Fig. 4). One case of a single coronary artery arising from the left coronary sinus was detected (0.07%). In this case, the RCA was absent, the CX was well developed and continued in the right AV groove as the right coronary artery (Fig. 5). In eight cases (0.53%), an anomalous CX originated from the right coronary sinus. It followed a retroaortic course in all cases (Fig. 6). Eight cases of anomalous RCA arising from the left coronary sinus were observed (0.53%). The RCA followed an interarterial course between the aortic root and the pulmonary artery in six cases and a retroaortic course in the remaining two cases (Fig. 7). In four cases (0.27%), the LMCA was absent and the LAD and CX originated separately from the left coronary sinus.

Discussion

The CCA has been a gold standard technique in order to visualize the coronary arteries for decades. In addition to being an invasive technique, absence of 3D imaging and soft tissue visualization are the main disadvantages of the CCA as oppose to the coronary MDCTA in detecting coronary artery anomalies [4]. The sensitivity of the MDCTA for detection of the proximal coronary artery stenosis varies between 80% and 90% with 4 and 16-slice scanners. 16-slice MDCTA permits a complete diagnosis for the coronary artery anomalies. It has been shown that MDCTA is superior to the CCA in determining the origin and proximal course of the anomalous coronary arteries [8-10]. Kacmaz et al. [3] issued a report about the 100% sensitivity of 16-slice MDCTA for detecting the coronary artery anomalies. On the other hand Shi et al. [8] reported a sensitivity of 100% for 16-slice MDCTA in detecting anomalous origin and course of the coronary arteries in their series whereas the sensitivity of CCA was only 53%. The accurate evaluation of the MDCTA requires awareness of the anatomy, normal variants and anomalies of the coronary arteries [4].

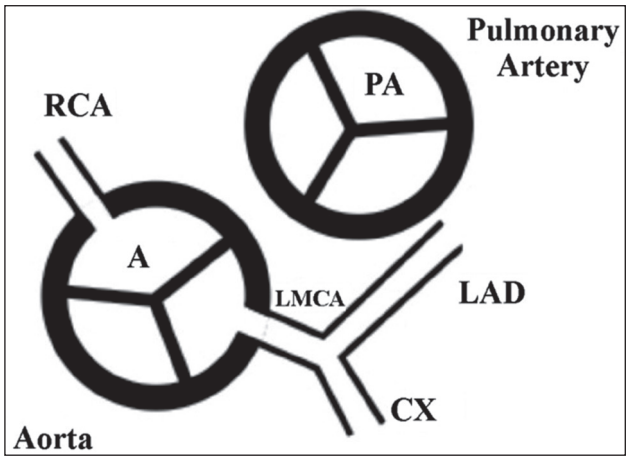
In the single coronary artery anomaly, only one coronary artery originates with a single ostium from the aorta. The prevalence of this extremely rare congenital anomaly is between 0.0024% and 0.44% [5]. The clinical results of a single coronary artery ranges from a normal life expectancy to the high risk for sudden death if a coronary artery follows an interarterial course between the pulmonary artery and aorta. Moreover, proximal stenosis of a single coronary artery may be fatal if there is an incapability to improve collateral vessels [7]. There were two cases of single coronary artery anomaly in our study (0.13%). In the first case, a single coronary artery was originating from the right coronary sinus and trifurcating into the LAD, CX and RCA. The LAD followed a prepulmonic course, the CX followed a retroaortic course and the RCA followed a normal course. In the other case, the single coronary artery was originating from the left coronary sinus and bifurcating into the LAD and CX. The RCA was absent. The CX was well developed and continued in the right AV groove as the right coronary artery. The LAD followed a normal course.

The anomalous origin of the left coronary artery from the pulmonary artery (ALCAPA) is a rare and serious congenital coronary artery anomaly. It has a reported prevalence of one in 300,000 live births [11]. In our study, ALCAPA was observed in only one case (0.07%). The symptoms of ALCAPA occur in infants and early childhood. Almost 90% of infants do not survive more than one year, if left untreated. Only a few patients reach to the adulthood. The most prevalent form of the disorder is Bland-White-Garland syndrome, in which the LMCA originates from the pulmonary artery and the RCA originates from the aorta [5] as in our patient. The imaging findings of ALCAPA consist of the LMCA originating from the posterior aspect of the pulmonary artery, a dilated and tortuous RCA and multiple dilated intercoronary collateral vessels [12]. These typical imaging findings were detected in our patient. The treatment of ALCAPA consists of surgical procedures.

The anomalous origin of the coronary artery from the opposite sinus is a possibly dangerous anomaly. Four patterns of anomalous origin may be detected with this anomaly; the RCA originating from the left coronary sinus (0.03%-0.17%), the LMCA originating from the right coronary sinus (0.09%-0.11%), the LAD or CX originating from the right coronary sinus (0.32%-0.67%) and the RCA or LMCA originating from the noncoronary sinus [13-15]. In our study, the anomalous origin of the coronary artery from the opposite sinus was observed in 21 cases (1.4%). An anomalous coronary artery may follow four common courses before resuming its normal position; interarterial course, retroaortic course, prepulmonic course and intraseptal course. An anomalous RCA arising from the left coronary sinus usually follows the interarterial course [13]. We observed eight cases of RCA originating from the left coronary sinus in our study (0.53%). The RCA followed an interarterial course in six cases (75%) and a retroaortic course in two cases (25%). An interarterial course has been published in 75% of the patients with an anomalous LMCA arising from the right coronary sinus [15]. The LMCA may also follow retroaortic, prepulmonic or intraseptal courses [14]. In our study, five cases of anomalous LMCA arising from the right coronary sinus were detected (0.33%). The LMCA followed an interarterial course in two cases (40%) and a retroaortic course in three cases (60%). The most prevalent course of the anomalous CX originating from the right coronary sinus is retroaortic [13]. We observed eight cases (0.53%) with this anomaly in all of which the course of the CX was retroaortic. The RCA or LMCA originating from the noncoronary sinus is a rare anomaly and usually has no clinical relevance [5].

In multiple ostia, the LMCA is absent and the LAD and CX originate separately from the left coronary sinus. The reported prevalence of this anomaly varies between 0.41% and 3.3% [16-19]. The corresponding prevalence in our study was 0.27%. The high takeoff of the coronary arteries means that the origin of the LMCA or RCA is over the junctional zone of the ascending aorta. Reported prevalence of this abnormality is 6% [5]. The multiple ostia and high takeoff of the coronary arteries are usually categorized as normal variants or minor anomalies. They are not associated with any hemodynamic abnormality, however they may pose technical difficulties during coronary artery catheterization and may increase the risks of complications and misdiagnosis [5, 16, 17, 18].

As a result, the complicated anatomy of the coronary arteries can be precisely defined by the MDCTA. It allows an exact and noninvasive definition of the congenital coronary anomalies. The MDCTA visualizes the origin and course of the anomalous coronary arteries better than the CCA and it is regarded as a guide to the interventional procedures or surgery to avoid possible complications. The awareness of the CT features of different coronary artery anomalies with knowledge of their clinical importance is essential in obtaining an accurate diagnosis.



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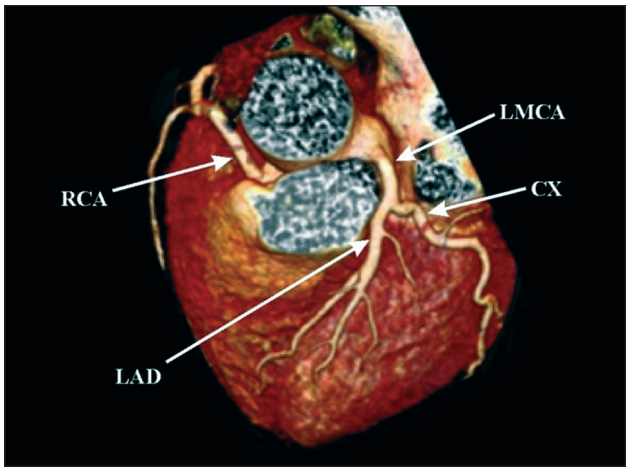

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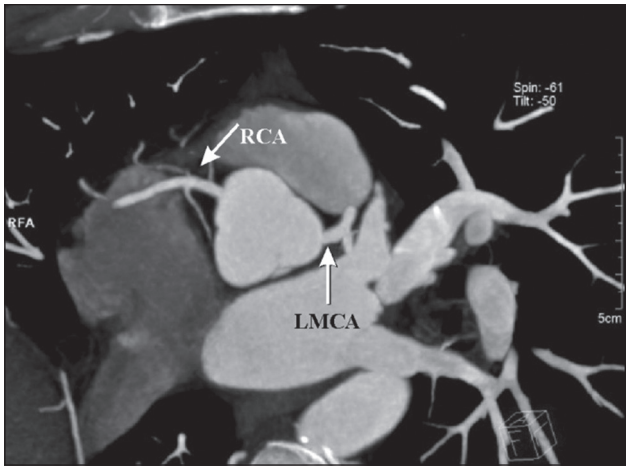

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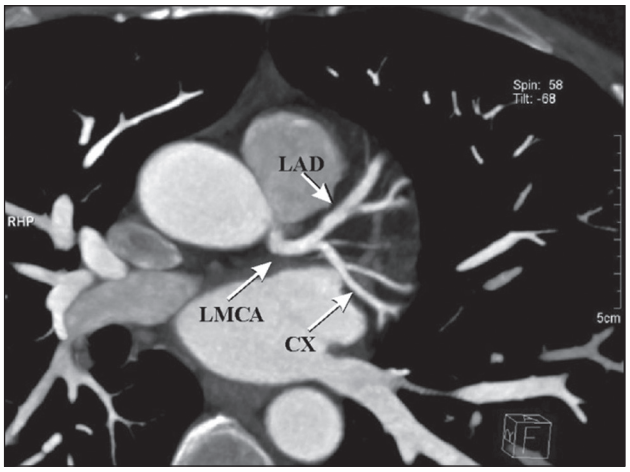

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Figure 1. a-d Normal anatomy of the coronary arteries. Schematic (a), volume-rendering technique (VRT) (b), maximum intensity projection (MIP) (c-d) images show the RCA arising from the right coronary sinus, the LMCA arising from the left coronary sinus and bifurcating into the LAD and CX. LMCA, the left main coronary artery; LAD, the left anterior descending artery; CX, the left circumflex artery; RCA, the right coronary artery; A, aorta; PA, pulmonary artery.

Table 1. Coronary artery anomalies

Anomalies of origin
High takeoff
Multiple ostia
Single coronary artery
Anomalous origin of coronary artery from the pulmonary artery
Anomalous origin of coronary artery from opposite sinus or noncoronary sinus with anomalous course (retroaortic, interarterial, prepulmonic, septal)
Anomalies of course
Myocardial bridging
Duplication of coronary arteries
Anomalies of termination
Coronary artery fistula
Coronary arcade
Extracardiac termination

Source: Ref. [7]

Table 2. Prevalence of anomalies of the coronary arteries

	Anomalies	Number of cases	% (1500 patients)	% (28 patients)
1	LMCA originating from the right coronary sinus In 3 cases LMCA had retroaortic course In 2 cases LMCA had interarterial course	5	0.33	17.86
2	Left coronary artery originating from the pulmonary artery (ALCAPA)	1	0.07	3.57
3	Single coronary artery originating from the right coronary sinus. It trifurcated into LAD, CX and RCA. LAD had prepulmonary, CX had retroaortic, RCA had normal course.	1	0.07	3.57
4	Single coronary artery originating from the left coronary sinus with absent RCA	1	0.07	3.57
5	CX originating from the right coronary sinus with retroaortic course	8	0.53	28.57
6	RCA originating from the left coronary sinus In 6 cases RCA had interarterial course In 2 cases RCA had retroaortic course	8	0.53	28.57
7	Absent LMCA with separate origin of LAD and CX	4	0.27	14.29

LMCA, the left main coronary artery; LAD, the left anterior descending artery; CX, the left circumflex artery; RCA, right coronary artery.

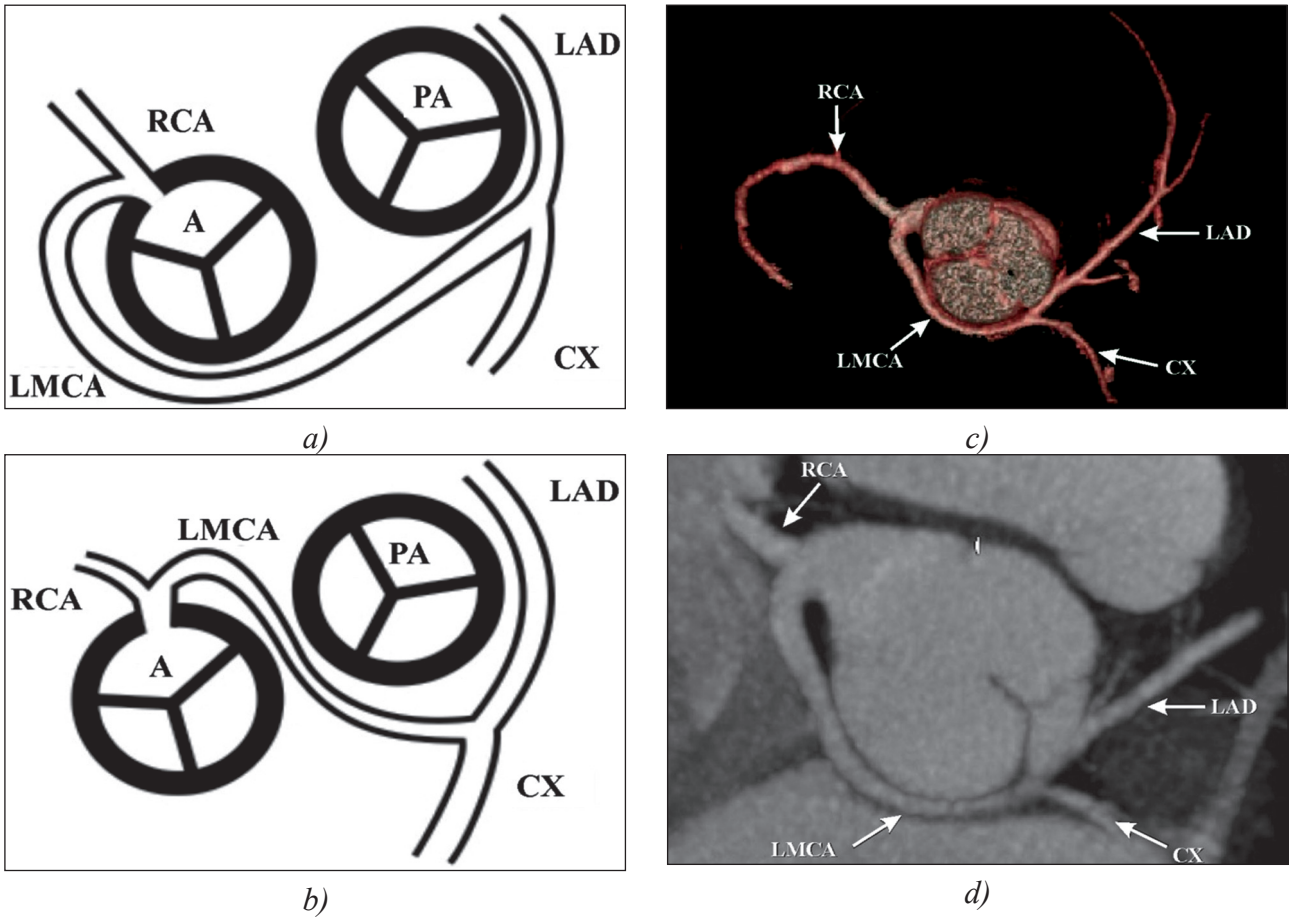
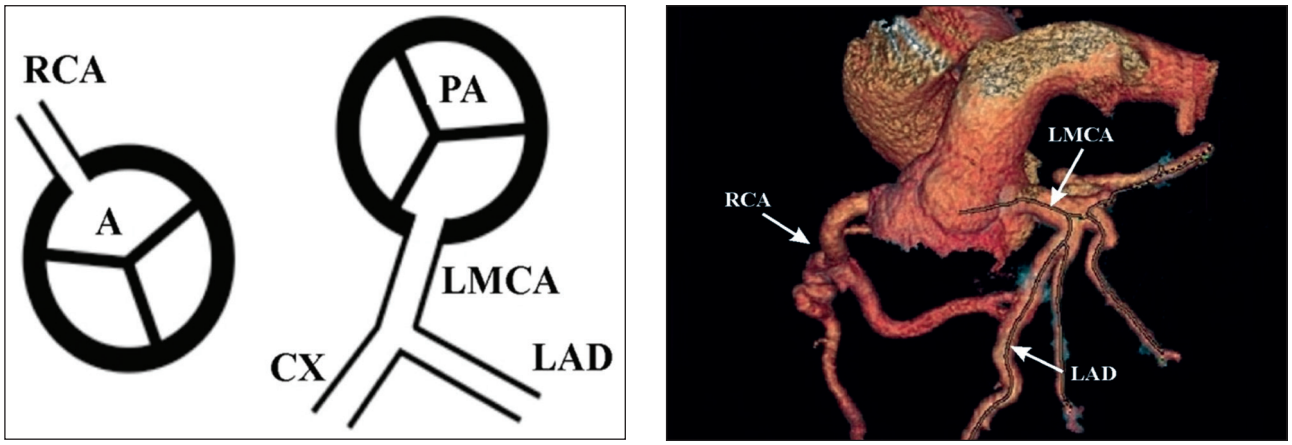
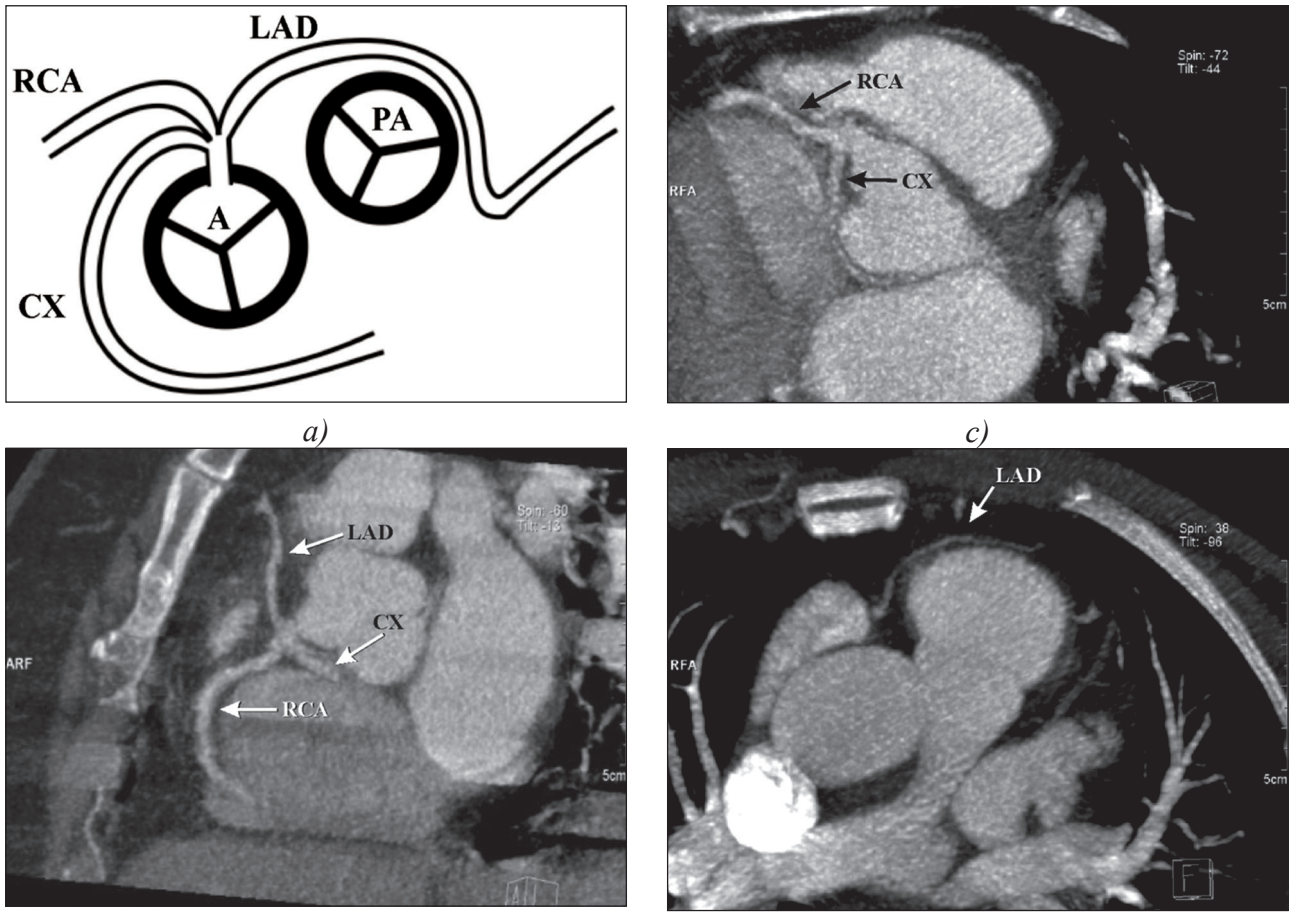


Figure 2. a-d. Anomalous origin of the LMCA from the right coronary sinus. Schematic images of the anomalous LMCA arising from the right coronary sinus with a retroaortic course (a) and with an interarterial course (b). VRT (c) and MIP (d) images of a 16 years old male patient with effort angina show the LMCA originating from the right coronary sinus and following a retroaortic course. LMCA, the left main coronary artery; LAD, the left anterior descending artery; CX, the left circumflex artery; RCA, the right coronary artery; A, aorta; PA, pulmonary artery.



a) b)

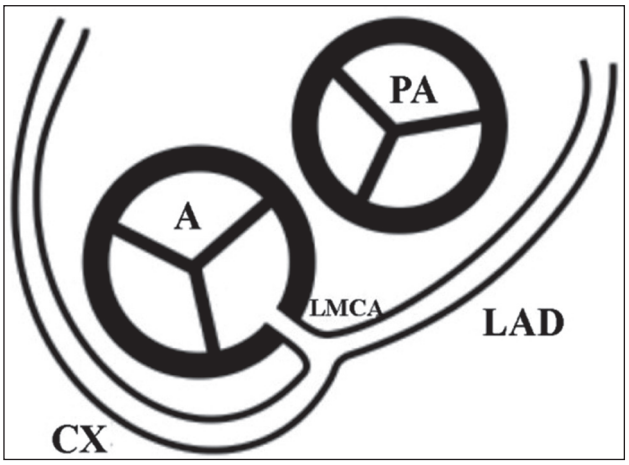
Figure 3. a-b. Anomalous left coronary artery arising from the pulmonary artery (ALCAPA). Schematic image (a) shows the LMCA originating from the posterior aspect of the pulmonary artery and the RCA arising from the right coronary sinus of the aorta. VRT image (b) of a 34 years old male patient with unstable rest angina shows the LMCA originating from the pulmonary artery with dilated and tortuous RCA arising from the aorta. LMCA, the left main coronary artery; LAD, the left anterior descending artery; CX, the left circumflex artery; RCA, the right coronary artery; A, aorta; PA, pulmonary artery.



a) c)

b) d)

Figure 4. a-d. Single coronary artery originating from the right coronary sinus. Schematic image (a) and MIP images (b, c, d) of a 53 years old female patient with effort angina show the single coronary artery originating from the right coronary sinus and trifurcating into the LAD, CX and RCA. LAD follows a prepulmonic, the CX follows a retroaortic and the RCA follows a normal course. The LMCA is absent. LMCA, the left main coronary artery; LAD, the left anterior descending artery; CX, the left circumflex artery; RCA, the right coronary artery; A, aorta; PA, pulmonary artery.



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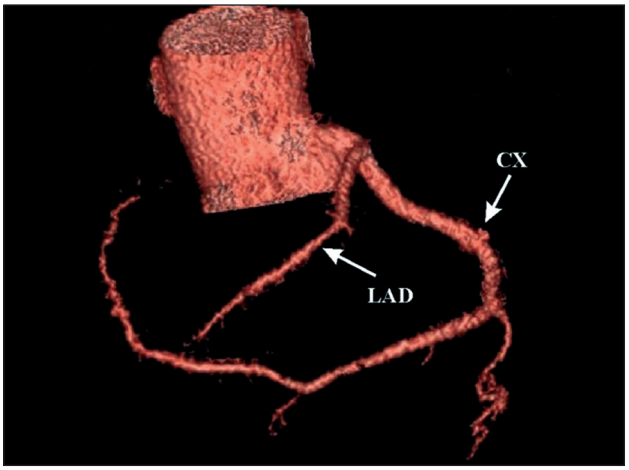

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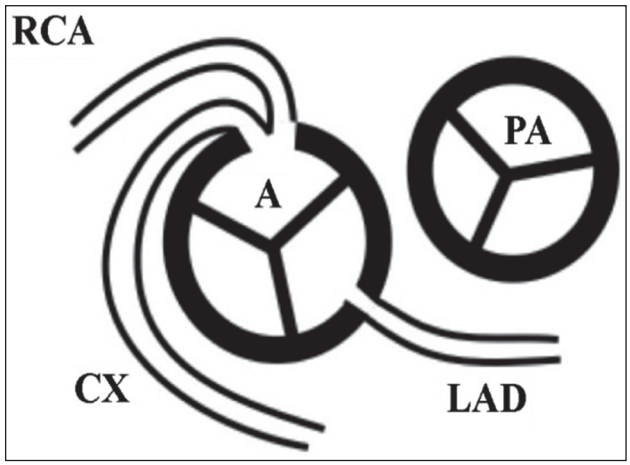
Figure 5. a-b. Single coronary artery arising from the left coronary sinus with absent RCA. Schematic image (a) and VRT image (b) of a 48 years old female patient with unstable angina show a single coronary artery arising from the left coronary sinus. The RCA is absent. The well developed CX continues in the right AV groove as the right coronary artery. The LAD has a normal course. LMCA, the left main coronary artery; LAD, the left anterior descending artery; CX, the left circumflex artery; RCA, the right coronary artery; A, aorta; PA, pulmonary artery.



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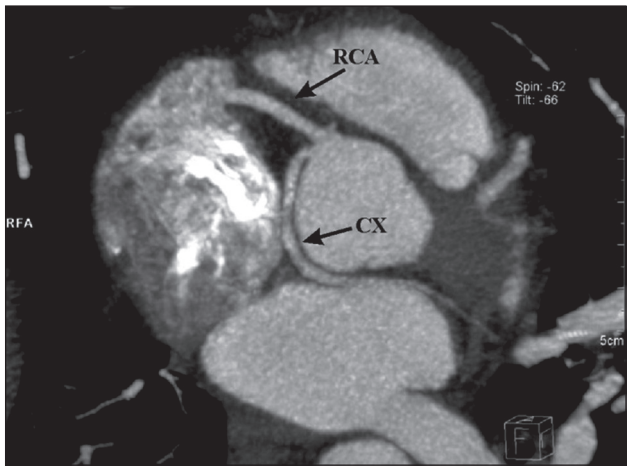

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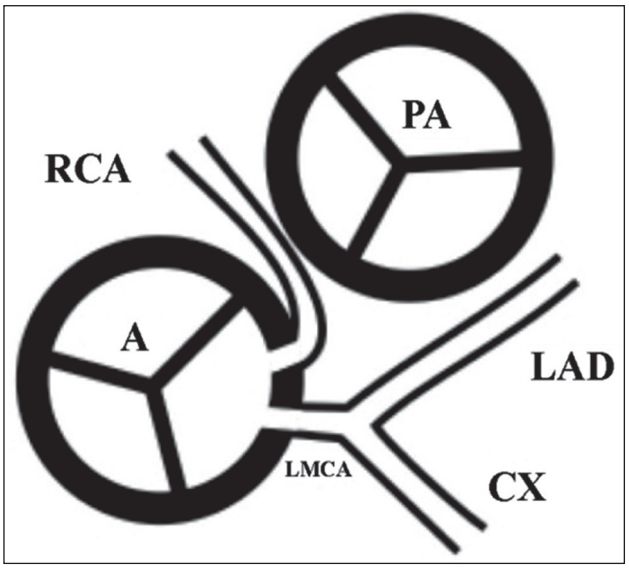
Figure 6. a-b. Anomalous CX originating from the right coronary sinus. Schematic image (a) and MIP image (b) of a 37 years old male patient with atypical chest pain show the anomalous origin of the CX from the right coronary sinus. The CX follows a retroaortic course. LMCA, the left main coronary artery; LAD, the left anterior descending artery; CX, the left circumflex artery; RCA, the right coronary artery; A, aorta; PA, pulmonary artery.



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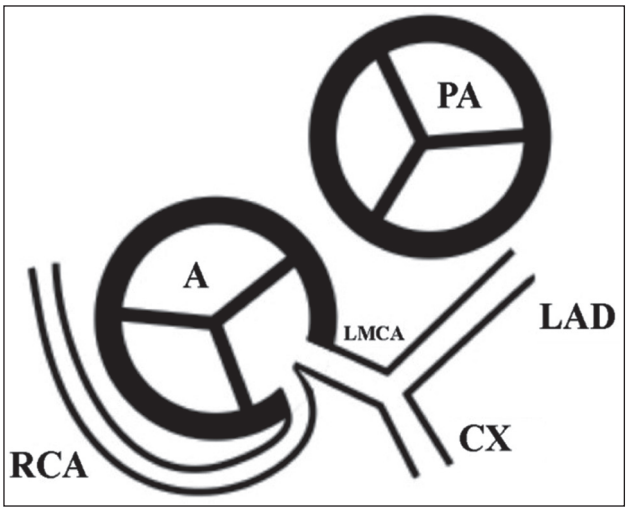

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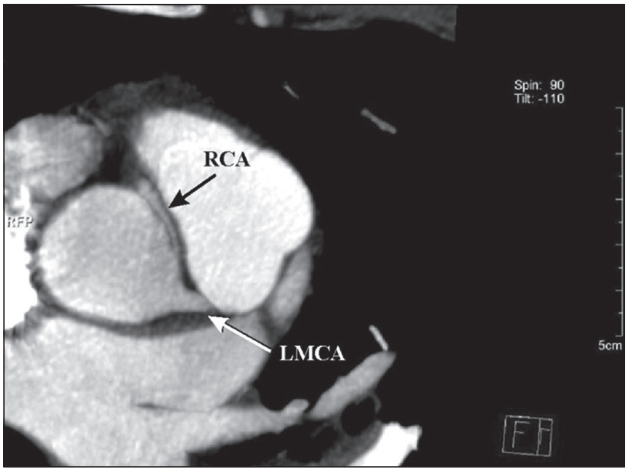

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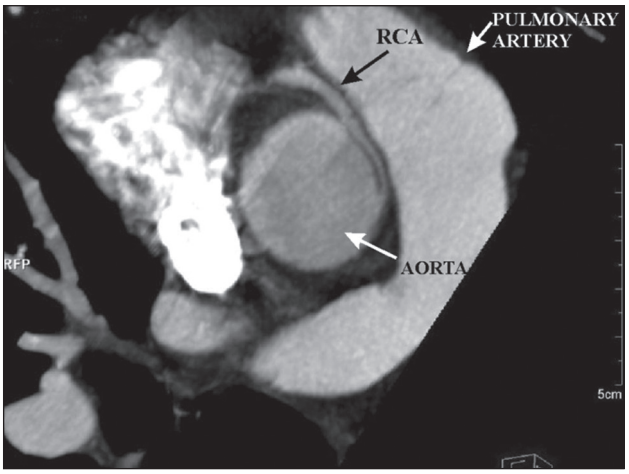

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Figure 7. a-d. Anomalous origin of the RCA from the left coronary sinus. Schematic images of the anomalous RCA arising from the left coronary sinus with an interarterial course (a) and a retroaortic course (b). MIP images (c-d) of a 39 years old male patient with exercise dyspnea and angina show the RCA originating from the left coronary sinus and following an interarterial course. The proximal RCA narrows between the aortic root and the pulmonary artery. LMCA, the left main coronary artery; LAD, the left anterior descending artery; CX, the left circumflex artery; RCA, the right coronary artery; A, aorta; PA, pulmonary artery.

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Functional disability and MRI findings in lumbar disc herniation

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Abstract

Introduction: Magnetic resonance imaging (MRI) is important diagnostic procedure in the low back pain (LBP), especially in the radiculopathy caused with disc herniation. The aim of this study was to determine how various anatomic impairments (size and location) of lumbar disc herniation and nerve compression are associated with patient reports of pain and functional disability.

Methods: The study included 134 patients, mean age 54,7 ± 10,5 years, with lumbar disc herniation and LBP, treated from October 2010 to January 2012 in the Clinic for Medical Rehabilitation, Clinical Center of Vojvodina. The presence of disc herniation and impingement of nerve structures were estimated by MRI. The duration, distribution and level of the patients' pain and disability, were rated by three guestionnaires: 1.Visual analog scale, 2. Pain Detect Test and 3. The Oswestry disability questionnaire. For assessment of the spinal flexibility and mobility were used tests according to Schober and Thomayer.

Results: Mean pain duration was 12,8 months. Pain radiation in the leg had 56 (42%) patients. The size of the herniation 3 mm or less had 33 (24,6%) and above 3 mm had 101 (75,4%) patients. Pain radiation was oftener and levels of the patients' pain and disability were significant greater in the patients with herniation size over 3 mm than less and with dorso-lateral and dorsal than lateral location ($p < 0.05$). Among patients, with pain radiation, 40 (71,4%) had MRI visible nerve root impingement (touching, deviation or compression) or thecal sac compression. These patients had significant higher values of all tests, compared with the group without affected nerve structures.

Conclusions: The size of disc herniation is partly important, but of a much greater impact on pain occurrence and functional disability are dorso-lateral location and nerve involvement. ***So*** the MRI findings, showing these changes, may be useful in diagnostic and therapeutic procedures.

Key words: Functional disability, MRI findings, Lumbar disc herniation

Introduction

Low back pain (LBP) is a common human ailment. It is estimated that over 70% of the population during the life will experience low back pain that will require medical attention and treatment. Important structures regarding possible pain sources are the intervertebral discs, zygapophyseal joints (also called facet joints), the ligaments and the muscles. As a principle of nociception, all structures in low back region that are innervated can cause low back pain. Among mentioned the most common causes of the LBP are degenerative changes and the lesions of the intervertebral discs. Intervertebral disc degeneration (IVDD) suggests a complex process influenced by genetics, lifestyle and biomechanics, which accounts for the development of LBP and lumbar radiculopathy, a major cause of musculoskeletal disability in humans (1, 2).

One of the IVDD manifestations is herniation of the lumbar spine discs, which is often associated with lumbar radiculopathy. For most pain-associated problems, it is difficult to predict which patients will develop chronic pain and functional disorders (1, 2, 3, 4).

Magnetic resonance imaging (MRI) is very important diagnostic procedure with the aim to find out the cause of the low back pain, especially in the form with associated radiculopathy caused with disc degeneration and disc herniation. It is also recognized that treatment, patient reliability and insurance questions ultimately depend largely on the detection of MRI-visible nerve involvement. But it is well known that sometimes MRI findings are not in the correlation with intensity of the pain and with physical, psychosocial and functional

disorders (5, 6, 7). Therefore, the MRI findings have to be regarded with caution taking into account that sometimes psychosocial factors predict functional disability due to disc herniation better than imaging. In these cases, where LBP cannot be explained with peripheral anatomy solely, central neural and psychophysiological, psychosocial mechanisms, and cognitive issues, as well as controlling movement are of greater importance (1).

On the other hand, a large study on 1043 Chinese subjects found a significant association between symptoms in LBP and MRI findings. Degenerative disc changes and herniation were twice as common in patients with severe back pain compared to subjects without back pain (8). Also the study of Chen JY. et al. found that MRI is useful diagnostic procedure in low back pain (9).

According clinial guidelines it should not routinely perform MR imaging in patients with nonspecific low back pain. Magnetic resonance imaging is indicated for patients with low back pain only if they have signs or symptoms of radiculopathy or spinal stenosis with severe progressive neurologic deficits or signs or symptoms that suggest a serious or specific underlying condition which are potential candidates for surgery or epidural steroid injection (1, 10).

As the relationship between MRI findings of discopathy with disc herniation and intensity of back and leg pain, functional status, health-related quality of life, and the objectively measured spinal mobility is often unknown, we decided to assess the relationship among these factors in a prospective study.

Methods

The study included 134 patients, 64 females (47,8%) and 70 males (52,2%), with lumbar disc herniation and LBP, treated from October 2010 to January 2012 in the Clinic for Medical Rehabilitation, Clinical Center of Vojvodina. Persons with diabetes and alcoholism were not included. The study was designed as a prospective and was conducted with the consent of patients and local ethic committee, as well as in concordance with the Helsinki Declaration. The presence of disc herniation was estimated by MRI of lumbar spine. Mean age of the patients was 54,7 ± 10,5 years (28 – 74). The duration, distribution, and level of the patients' pain and disability were rated and recorded by using experience of disease by three guestionnaires: 1. (Visual analog scale (VAS), for assessment of pain intensity 2. Pain Detect Test (PDT) for assessment of character, timing, radiating, and neuropathic component of the pain and 3. The Oswestry disability questionnaire (version 2.1) for pain intensity, patients' ability for selfcare and social life assessment.

For assessment of the spinal flexibility and mobility were used tests according to Schober and Thomayer.

In addition, disability concerning physical work, during the past four weeks, was assessed in men.

Assessment based on MRI

Participants underwent standardized lumbar magnetic resonance imaging using a 1.5-T scanner. MRI scans were carried out according to the protocol prescribed by the radiologist with included T1- and T2-weighted and also axial T2-weighted images of the lumbo-sacral spine. Each MRI scan was assessed according to a standardised protocol and classified for the presence or absence of abnormalities: disc herniation (protrusion, extrusion or sequestration) or nerve root impingement (touching, deviation or compression) at any of the four spinal levels from L2/L3, L3/L4, L4/5 and L5/S1 or thecal sac compression. For the evaluation of the disc herniations and their location and graduation were used proposed criteria by Bertilson B. et all. (11) which implies focal protrusion of the disc through a defect in the annulus fibrosus into the spinal canal (central or posterior), posterolateral - foraminal and lateral space. According the size of the disc protrusion the patients were classified into two groups: 3 or less than 3 mm (≤ 3 mm) and more than 3 mm (> 3 mm). Nerve root impingement was evaluated using the grading system of Pfirrman *et al.* and was characterized as "no impingement," "touching" (contact), "displaced" (deviation), or "compressed" (12).

Statistical analysis was carried out with STATA version 11 software. Data were expressed as means ± standard error. The statistical significance of the difference between the means was performed with Student's independent test, and due to assumption of abnormal distribution of variables were appli-

ed nonparametric tests (Mann-Whitney). Poisson regression was used to estimate prevalence ratios (PRs) with their 95% confidence intervals (CIs). When investigating correlation between functional measures and pain to type of discopathy Pearson's correlation was performed. Values of $p < 0.05$ were regarded as statistically significant.

Results

The results of the examination are shown in the following Tables. Their detailed explanation and comparison with literature data are presented in chapter discussion.

Table 1. Patient characteristics (n = 134)

Basic characteristics	Median or %
Age, years (range)	54,7 (28 - 74)
20-39 years	30 (22,4%)
40-49 years	41 (30,6%)
50-74 years	63 (47,0%)
Gender – No (%)	
females	64 (47,8%)
males	70 (52,2%)

Table 2. Pain / discomfort duration and radiation

Pain / discomfort in low back region	n (%)
Duration <2 months	26 (19)
Duration 2-12 months	32 (24)
Duration 1-2 years	18 (14)
Duration > 2 years	58 (43)
Mean pain duration (months)	12,8
Presently pain radiation	56 (42)
-into the buttock	25 (19)
-below the buttock	31 (23)

Table 3. Disc herniation size - MRI results

Size of the herniation	n (%)
herniation ≤ 3 mm	33 (24,6)
herniation > 3 mm	101 (75,4)

Table 4. Radiation of the pain and size of herniated disc

Pain radiation (n=56)	herniation > 3 mm	herniation ≤ 3 mm	p
Into buttock (n=25)	21 (84%)	4 (16%)	< 0.05
Below knee (n=31)	28 (90%)	3 (10%)	< 0.05

Table 5. Characteristics of disc herniation over 3 mm of the diameter (n = 101)

Levels	n (%)
L2-L3	6 (6,0%)
L3-L4	18 (18%)
L4-L5	45 (44%)
L5-S1	32 (32%)
Two or more levels	45 (44%)
Herniation type	
Protruding	27 (27%)
Extruded	67 (66%)
Sequestered	7 (7%)

Table 6. Pain and functional disability tests in all examinated patients (n = 134)

Tests	min - max	mean (SD)
Pain Detect Test (PDT) score	11 - 36	22,5 (8,7)
Visual analog scale (VAS)		
- now at this moment	5 - 9	7,2 (1,6)
- the strongest pain in last 4 weeks	4 - 8	7,5 (1.4)
Oswestry disability score (ODS)	25 - 43	34,8 (10,3)
Thomayer test (cm)	11 - 34	20,4 (6,8)
Schober test (cm)	2 - 4,5	3,4 (0,7)

Table 7. MRI visible nerve and thecal sac impingement / compression among the patients with pain radiation (n=56)

Impingement	n
Impingement not found	16 (28,6%)
With visible impingement	40 (71,4%)
-nerve touching	4
-nerve deviation	14
-nerve compression	16
-thecal sac compression	6

Table 8. Size of disc herniation and results of tests

Tests	herniation size > 3 mm (n=101)		herniation size ≤ 3 mm (n=33)		p
	min - max	mean (SD)	min - max	mean (SD)	
Pain Detect Test (PDT) score	l7 - 36	33,5 (11,6)	11 - 20	16,3 (5,4)	<0.05
Visual analog scale (VAS)					
-now at this moment	6 - 9	8,7 (1,3)	5 - 7	5,1 (1,6)	<0.05
-the strongest pain in last 4 weeks	7 - 8	8,9 (1.7)	4 - 7	5,2 (1,5)	<0.05
Oswestry disability score (ODS)	34 - 43	41,7 (9,7)	25 - 38	28,2 (11,7)	<0.05
Thomayer test (cm)	16 - 34	24,7 (7,1)	11 - 23	16,8 (6,7)	<0.05
Schober test (cm)	2 - 4	3,1 (0,7)	3 - 4,5	3,9 (0,8)	<0.05

Table 9. Disc herniation location and results of tests

Tests	lateral (n=14)		dorsal (n=48)		dorso-lateral (n=72)		p
	min-max	mean (SD)	min-max	mean (SD)	min-max	mean (SD)	<0.05
PDT* score	9-17	13,1 (4,7)	14-33	23,4 (10,7)	22-36	34,2 (9,4)	<0.05
VAS**							
-now	4-7	5,1 (1,6)	6-8	7,2 (1,7)	7-9	8,7 (1,2)	<0.05
-last 4 weeks	5-7	5,2 (1.5)	6-8	7,1 (1,2)	7-9	8,9 (1.3)	<0.05
ODS***	16-22	18,3 (4,5)	27-38	32,1 (5,1)	36-43	42,2 (5,5)	<0.05
Thomayer test	11-18	15,4 (6,7)	17-30	23,1 (6,8)	19-37	27,4 (8,8)	<0.05
Schober test	3-4,5	3,5 (0,7)	3-4	3,3 (0,5)	2,5-4	2,9 (0,7)	n.s.

PDT Pain Detect Test*
*VAS** Visual analog scale*
*ODS*** Oswestry disability score*

Table 10. Nerve root impingement, thecal sac compression and test results in the group with pain radiation (n=56)

Impingement/ compression	PDT* score	ODS**	VAS***	VAS****	Thomayer	Schober	p
	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	
No impingem. (n=16)	10,1 (2,3)	16,4 (6,3)	4,3 (1,1)	4,4 (1,3)	14,3 (5,5)	3,8 (0,6)	-
Touching (n=4)	21,7 (4,5)	39,7 (7,3)	7,6 (1,4)	7,8 (1,8)	21,2 (6,7)	3,2 (0,7)	< 0.05
Deviation (n=14)	28,5 (11,2)	41,2 (8,9)	7,7 (1.3)	7,9 (1,3)	28,3 (8,2)	3,0 (0,6)	< 0.05
Compression (n=16)	34,4 (10,3)	42,2 (7,3)	8,7 (1,5)	8,9 (1,6)	33,4 (8,9)	2,7 (0,7)	< 0.05
Thecal sac compression (n=6)	32,1 (5,6)	44,1 (4,2)	8,4 (1,7)	8,3 (1,4)	23,2 (6,8)	3,2 (0,4)	< 0.05

*PDT * Pain Detect Test*
*ODS ** Oswestry disability score*
*VAS *** Visual analog scale in this moment*
*VAS **** Visual analog scale in last 4 weeks*

Table 11. MRI visible nerve root impingement - thecal sac compression and test results in the group with pain radiation (n=56)

Impingement/ compression	PDT* score	ODS**	VAS***	VAS****
	PR (95% CI)	PR (95% CI)	PR (95% CI)	PR (95% CI)
No impingem. (n=16)	1 -	1 -	1 -	1 -
Touching (n=4)	1,4 (0,8-1,9)	1,5 (0,8-2,5)	1,4 (0,8-2,1)	1,5 (0,8-2,2)
Deviation (n=14)	1,5 (0,9-2,4)	1,6 (0,9-2,9)	1,5 (1,0-2,4)	1,6 (0,9-2,8)
Compression (n=16)	1,7 (0,9-3,0)	1,7 (1,0-2,9)	1,6 (0,9-2,9)	1,7 (0,9-2,9)
Thecal sac compression (n=6)	1,6 (1,1-2,5)	1,6 (1,0-2,8)	1,5 (0,9-2,5)	1,6 (0,8-2,7)

*PDT * Pain Detect Test*
*ODS ** Oswestry disability score*
*VAS *** Visual analog scale in this moment*
*VAS **** Visual analog scale in last 4 weeks*

Table 12. Size of disc herniation and physical job ability / disability in last four weeks – men (n=70)

Lowering of ability (%)	Size of the disc herniation		p
	> 3 mm	≤ 3 mm	
	(n=48)	(n=22)	
< 20%	2 (2,8%)	6 (8,6%)	< 0.05
20-39%	12 (17,1%)	11 (15,7%)	n.s.
40-59%	14 (20,0%)	5 (7,2%)	< 0.05
60-80%	16 (22,9%)	-	< 0.05
> 80%	4 (5,7%)	-	< 0.05
unable to work	13 (18,6%)	-	< 0.05

Table 13. Location of disc herniation and physical job ability / disability in last four weeks - men (n=70)

Location of herniation	Lowering of working ability (%)					
	< 20%	20-39%	40-59%	60-80%	> 80%	unable to work
lateral (n=12)	3(25%)	5(41%)	2(17%)	2(17%)	-	-
dorsal (n=23)	3(13%)	5(22%)	5(22%)	5(22%)	1(4%)	4(17%)
dorso-lateral (n=35)	2(6%)	4(10%)	8(23%)	9(26%)	3(9%)	9(26%)

Discussion

MRI has emerged as the diagnostic method of choice for assessing spine disorders and especially for detecting nerve involvement (8). However, scientifically, the agreement between MRI-visible nerve involvement and other diagnostic methods remains speculative, except the fact that severe MRI-visible nerve involvement in the lumbar spine is associated with radiation of the pain into the leg, which is clinically considered as a sign of nerve involvement (11). In our study the radiation of the pain into the leg was registered in 56 (42%) patients (Table 4). Out of them in 40 (71%) patients were found MRI-visible nerve root and thecal sac impingements i.e. compressions but in 16 (29%) patients these changes were not registered (Table 7). Our results are similar to the results of Bertilson BC, et al. (11). They found the mean specificity of MRI-visible nerve involvement ranged from 61-77%. But positive and negative predictive values of MRI-visible nerve involvement in segment L4-5 ranged from 22-78% and in other segments 28-56% (11). Our findings are consistent with these results. On the other hand, all patients with MRI-visible nerve root impingement had also pain radiation into the leg. One large study also found a significant association between symptoms in LBP and MRI findings (8). But, some studies and autohors suggest that the MRI findings and abnormalities, examined in epidemiological research, are not major predictors of outcome in patients with LBP and they give no support to the use of MRI findings as a standard diagnostic procedure for LBP (5, 6). The explanation of these results could

be reliability of MRI finding or patient's subjective assessment of the pain radiation (15).

Endean A. et al. found that MRI findings of disc protrusion and nerve root displacement / compression are associated with LBP, but individually, and none of these abnormalities provides a strong indication that LBP is attributable to underlying pathology (5).

Though national guidelines discourage the use of MRI in non-specific LBP and recommend reserving it only for the investigation of severe or progressive neurological deficits or for those cases in which serious underlying pathology is suspected, MRI also has an acknowledged role in planning surgical management in cases of radiculopathy and spinal stenosis (16).

The results of our study showed the association between MRI-visible nerve root impingement and pain radiation or discomfort into the leg as a clinical sign of nerve impingement (Table 7). The most frequent localisations of disc herniations over 3 mm of diameter were in segment L4-L5 in 45 (44%), and L5-S1 in 32 (32%) patients. With two or more levels of disc herniations were 45 (44%) patients (Table 5). Similar results are found in the study of Endean A. et al. (5).

One of the aim of our study was to evaluate the agreement between MRI-visible disc herniation of the lumbar spine, including the size of herniation and its localisation, and nerve involvement as well as pain characteristics and functional disability of the patients.

Results of our investigations showed significant influence of the herniation size (over 3 mm) and also of the herniation location (dorso-lateral and dorsal location) on pain drowing and functional disability (Table 8 and Table 9). Out of 101 patients with herniation size over 3 mm, 49 (48,5%) had pain radiation into the leg (21 into buttock and 28 below knee). On the other hand, out of 33 patients with herniation size less than 3 mm, 7 (21,2%) had also pain radiation (4 into buttock and 3 below knee), but their location was dorso-lateral/foraminal. Therefore, in the evaluation should be taken into account both of them, the size and the location of the herniation (13, 14). Of course, the big lateral herniation will have a particularly large impact on the occurrence of symptoms and disability. Similar results were obtained by other authors (13).

Also the great lateral herniation may affect the nerve through indirect pressure on surrounding tissue but the pressure will be relatively quickly reduced due to herniation resorption.

The score results of Pain Detect Test, Visual analog scale and results of Oswestry, Thomayer and Schrober tests were significant greater in the group with herniation size over 3 mm (Table 8). But it is visible that in this group, if minimal values of the score were taken into account, there were a few patients, with not so bad results. On the other hand, in the group with herniation size less than 3 mm, several patients were with bad results, having test scores about maximal values, similarly to the patients in the group "over 3 mm" (Table 8). These discrepancy between herniation size and syptomatology could be explained not only by different location and nerve root involvement, but also by the tendency to somatize the troubles and presence of psychosocial factors. Sometimes psychosocial factors predict functional disability due to disc herniation better than MRI imaging (7, 11). Also McNeel P, et al. found that disabling LBP was more strongly predicted by poor mental health, tendency to somatize and psychoisocial factors than by found pathoanatomical changes (6). Endean A. et al. found that the MRI-visible nerve involvement can be less prevalent than clinical and pain findings of nerve involvement (5). Sometimes the zygapophysial (facet) and sacroiliac joint can be responsible for back pain, although with less frequency than the disc (7).

Bertilson BC, et al. found that clinical findings correlate well with MRI findings, but all MRI abnormalities need not have a clinical significance. The presence of centrolateral disc protrusion and extrusions with gross neural foramen compromise and visible nerve involvement, associated with distal leg pain, are considered sign of nerve involvement (11).

In our study, when MRI findings were classified according to the MRI visible nerve root impingements and their influence on pain and its characteristics as well as on the results of PDT, ODS and VAS, the prevalence ratios (PRs) for highest vs. lowest categories was 1.7 with their 95% confidence intervals (CIs) between 1.0 - 2.5 (Table 11). Comparing the results of the group without impingement, taken as a basal values (PR=1), with findings of the

groups with nerve impingement it is visible that PRs (with 95% CIs) are growing. The greatest PRs of the tests were 1,6 – 1,7 in the group of the patients with nerve compression. These results are in concordance with studies that examined the prevalence of nerve root impingement and its relation to LBP symptoms, showing that most commonly graded MRI appearances were according to whether there was contact with a nerve root, or displacement or compression. The repeatability of such classification was good (5, 6, 8). In the cases when disc herniations were in two or more levels, the size and location of disc herniation as well as nerve impingement were also of crucial importance.

The influence of the size and location of disc herniation in the ability to work has been evaluated only in men because they are much more engaged in physical work than women. Among the patients who had a size of disc herniation more than 3 mm unfit for the job was 18,6% (Table 12). But location of disc herniation had greater influence to lowering of physical job ability than the size of herniation. In the group patients with dorso-lateral localisation of disc herniation 26% patients were unable to work (Table 13). Our results are consistent with the results of Bertilson BC, et al. (11).

But sometimes socioeconomic characteristics can be markedly different between patients receiving and not receiving workers' compensation (17). In some patients, besides the mentioned, also other psychosocial factors may affect the ability to work, for example tendency to somatise, anxiety, depression, fear avoidance of work and back pain related stresses (18, 19, 20, 21, 22). Biopsychosocial multidisciplinary interventions targeting these psychosocial variables may lead to improved quality of life and working capacity as well as healthcare costs in the working population (23, 24, 25), but this is not the theme of this study.

Conclusions

The results of our study showed that there are coherence and correlation between MRI-visible disc herniation and intensity and character of pain and functional ability / disability in patients with lumbar disc herniation. Of importance are the size of herniation and especially its localisation and nerve involvement.

The size of disc herniation is partly important, but of a much greater impact on pain occurrence and functional disability are dorso-lateral location and nerve involvement ***and so the*** MRI findings, showing these changes, may be useful in diagnostic and therapeutic procedures.

But it should be taken into account that sometimes MRI changes are not recognized or identified as visible causal factors. Also in some cases a significant impact on the intensity of symptoms and functional disability have psychological and psychosocial factors and this will be the subject of our further studies.

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Competing interest

The authors declare that they have no financial or personal relationship with people or organizations that could inappropriately influence this work.

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Pediatric traumatic brain injury management

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Abstract

Traumatic brain injury is described as a blow to the head or a penetrating head damage which disturb the normal function of the brain. Traumatic brain injuries, in children are common and sometimes are powerful in threatening the life and are leading causes of acquired disability and death. Traumatic brain injury is responsible for nearly 1.4 million injuries and 52 000 deaths annually in the United States.

Therefore, in this paper we reviewed the new and recent advances about the management and neuromonitoring in pediatric traumatic brain injury.

Key words: Traumatic brain injury, pediatrics, management

Introduction

Pediatric traumatic brain injury (TBI) is one of the main reasons of acquired disability and death, with the highest combined rates of TBI-related emergency room visits, needing an emergency evaluation in hospitals, and deaths (1). Checking intracranial pressure (ICP) and cerebral perfusion pressure (CPP), repeat neurological evaluations, computed tomography (CT) scanning and vital signs are thought the suitable and might be vital section of care following severe pediatric TBI (2).All recent epidemiological researches from Western Europe (3), northern Europe (4, 5, 6), north Africa (7), and the United States (8, 9, 10), establish that TBI remains an important public health burden for children and their families (11-16). TBI is responsible for 1.4 million injuries and 52000 deaths in the United States of America; according to annually reports (17). Motor vehicle crashes, falls, and assaults are the main source of brain damage and injury (18). Brain injury is common phenomenon in childhood. Five times as many children will die due to brain damage than from childhood malignancy (19).

Neurological diseases involving both primary neurological diagnoses and neurological complications of critical disorders are usually defined in the pediatric intensive care unit (PICU). A study, reported that almost one quarter of patients admitted to the PICU are predisposal to be at risk for acute neurological injury (showing the importance)(20).

PICU admitted-patients with neurological injury are showed to have higher mortality, more long-term morbidity, and longer length of in hospital stay (21).

An investigation revealed that more than half of the patients who died in a tertiary care center PICU had an acute brain injury. In most of these cases brain injury was considered to be the proximate reason of death (22). New strategies for diagnosis and treatment of lung, heart, kidney, and infectious diseases have dramatically decreased mortality and morbidity rates in the PICU section of the hospitals, the relative contribution of neurological disorders to mortality and long-term morbidity following critical diseases has increased (23).

Hence, in this article we studied the previous and new advanced methods of pediatric traumatic brain injury managements.

General management

To minimize the expansion of injury in the vulnerable brain, any additional insult must be avoided. The most appropriate management is indicated in table 1 (24-27).

Table 1. Preferential indications and precautions needed for the application of second-tier therapies for intracranial hypertension (24-27).

Therapeutic modality	Potential indications	Particularities
Aggressive hyperventilation (pCO2 <30mmHg)	Short-term use for refractory ICHT with normal or high CBF	Evaluate the preservation of CBF with transcranial Doppler (diastolic velocity >25 cm/s and pulsatility index <1.4) or brain oxygenation monitoring (pbtO2 >15mmHg)
High-dose barbiturates	Refractory ICHT in hemodynamically stable cases	Monitor EEG patterns for burst suppression Anticipate cardiovascular depression
Therapeutic hypothermia	No indication other than prevention of hyperthermia (T>38.5C)	
Decompressive craniectomy	Refractory ICHT in cases with reversible brain injury	Anticipate short-term complications (controlateral subdural effusion, seizures, and hydrocephalus)

CBF, cerebral blood flow; EEG, electroencephalographic; ICHT, intracranial hypertension; pbtO2, brain tissue oxygenation.

Hemodynamic objectives

Hypotension and hypoxia are mostly responsible for secondary brain injury at first six hours. Hypotension is very important (28) and shows that primary evaluation of blood pressure play a crucial role in management and outcomes and so patients' survival. Pediatric guidelines for the management of TBI which was published in 2003 suggested avoiding hypotension (systolic blood pressure <5th percentile) (29). Management of hypotension initiates with volume repletion (30). The commonest and most popular resuscitative fluid is Normal saline, but also the choice of fluids is still controversy (31).

Ventilatory objectives

Patients with severe TBI need an established airway and invasive mechanical ventilation; hypoxia should be avoided (29) and normocapnia (paCO2 36–45mmHg) maintained (29).

Sedation

Sedatives and analgesics are commonly prescribed to reduce cerebral metabolic requirements, consequently decreasing cerebral blood volume and ICP. These managements are individually changeable, taking into account the potential deleterious impacts, especially on blood pressure and ICP.

Hematologic disorders

Anemia usually happens after TBI. Coagulopathy frequently happens in the first few hours and up to 5 days after TBI, with a direct correlation with trauma severity and outcomes which should be considered (32). Coagulation disorders should be treated before ICP catheter placement.

Glycemic control

Glycemic control is subject of investigations and controversial in the context of neurocritical care. In the first 48 hours after TBI, hyperglycemia is being detected in 34% of children, even in the absence of glucose infusion (33). Hyperglycemia must be managed with proposed thresholds varying between 8 (34) and 10 mmol/l (35).

Neuromonitoring

Continuous evaluation of ICP and CPP in children with severe TBI is generally confirmed and suggested (36), despite low level of evidences. Minimalist objectives for ICP (<20mmHg) and CPP (>40mmHg) have been reported and an age-associated continuum for CPP levels (40–65mmHg) has been advised and defined (37). CPP of less than 45mmHg has been noted and established to have minus effects in children below 2 years of age (38). Optimal CPP and ICP values are not well defined and differ based on the type of injury and autoregulation status of the cases. Despite controlling of ICP and CPP according to the defined thresholds, many child patients have revealed the evidence of brain hypoperfusion with poor results (39,40).

Secondary Injury

One of the aims of primary management of TBI in children is to minimize secondary injury of the brain tissue. One of the main notable sources of secondary damage is cerebral ischemia and hypoxia (41). Finding cerebral ischemia with real-time continuous monitoring has been reported to be elusive. Adherence to ICP- and CPP-driven management guidelines does not assurance the avoidance of cerebral ischemia or hypoxia (42, 43). Cerebral blood flow threshold for cerebral ischemia in the nontraumatized brain has been indicated at 18 mL/100 g/min, and, when cerebral blood flow comes below 10mL/100 g/min, the result is cerebral infarction (44, 45).

Neuroimaging as a potential surrogate endpoint

Computerized tomography is a standard of care for children with TBI (46). But all centers don't have such equipments.Physicians are utilizing magnetic resonance imaging more frequently in such patients due to its higher sensitivity for the detection of brain damage and its potential ability to predict outcome (47-51).

Novel electrophysiology to evaluate coma in children with traumatic brain injury

Recently, synchrony of the electroencephalogram (EEG) has been investigated in these cases (52, 53). Patterns of EEG synchrony associates with the site of damage on neuroimaging and are related with functional outcomes.

Conclusion

Neuromonitoring technology is still at an early stage in pediatric TBI. These improvements have provided the possibility of true multimodal monitoring for useful treatments. In this regard, more investigations are required to determine whether these modalities and procedures in technology with noninvasive monitors will allow early and reliable diagnosis of reversible secondary brain insults. But, using clinical functional neuromonitoring would help clinicians to evaluate the managements in hospitals.

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D-dimer test to exclude left atrial appendage thrombus in patients with persistent atrial fibrillation

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Abstract

Background: Currently there is no routinely used noninvasive biochemical test to help detect left atrial appendage thrombus (LAA) in patients with persistent atrial fibrillation (AF). In this study we aimed to investigate the potential use of plasma D-dimer levels for exclusion of LAA thrombus in persistent AF patients.

Methods: Sixty-five non-anticoagulated patients with persistent AF (37 men, 28 women, mean age 56.89±11.03), scheduled for direct current cardioversion were included in the study. All patients were examined by transesophageal echocardiography (TEE) followed by blood sample withdrawal for plasma D-dimer measurement. TEE was accepted as the gold standard for detection of atrial thrombus.

Results: TEE detected LAA thrombus in 19 patients. Hypertension, mitral stenosis and D-dimer level (dichotomized as high/low) were independent predictors of LAA thrombus. D-dimer cut-off value was set at 1.60 μg/ml based on ROC analysis. At this cut-off D-dimer had 97% negative predictive value, while sensitivity, specificity, and positive predictive values were 95%, 78% and 64%, respectively.

Conclusion: Elevated plasma D-dimer is not a sufficient criterion to predict LAA thrombus in AF patients, without TEE examination. However, normal range D-dimer levels can exclude LAA thrombus with high sensitivity and negative predictive value.

Key words: Atrial fibrillation, D-dimer, left atrial appendage thrombus.

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, its prevalence increasing with advanced age [1]. Stroke, thromboembolism, and congestive heart failure (CHF) are important causes of morbidity and mortality in patients with AF. In Framingham study, risk of stroke was increased by 5-fold in AF patients, and 17-fold in AF patients with rheumatic heart disease [2]. Treatment goals of AF are heart rate control, rhythm control, and prevention of thromboembolism [3]. Treatment decision is based on symptom severity, age, clinical characteristics (associated cardiovascular disease or other medical conditions) and duration of AF [3]. Early rhythm control is important in symptomatic patients with worsening heart failure. Similarly, younger patients with primary AF would benefit from early rhythm control, since atrial remodeling over the long-term would prevent later restoration of sinus rhythm and lead to permanent AF. Restoration of sinus rhythm by elective direct current (DC) cardioversion is a therapeutic option in qualified patients with persistent AF. Since cardioembolic ischemic strokes resulting in severe loss-of-function originate primarily from thrombus formation in the left atrial appendage (LAA), transesophageal echocardiography (TEE) is performed to exclude LAA thrombus. TEE is a sensitive and specific tool for evaluating LAA function and detecting thrombus formation [4, 5]. However, although minor, TEE does harbor a certain risk of morbidity. In addition, TEE is not sensitive enough to detect small thrombi, less than 2 mm in size.

Thrombus formation and degradation depends on the equilibrium between thrombogenesis and fibrinolysis. Plasma D-dimer is a fibrinolysis end-

product. Contrary to fibrin degradation products derived from fibrinogen and fibrin, D-dimer is a specific cross-linked fibrin derivative [6]. D-dimer levels increase as a result of fibrin production and plasmin-mediated degradation. D-dimer is a reliable laboratory test to show coagulation activity and when used together with clinical scoring systems, it is an effective diagnostic test for venous thromboembolic diseases such as deep vein thrombosis and pulmonary embolism [7, 8].

Currently there is no noninvasive test to detect LAA thrombus in persistent AF. In this study we investigated the role of plasma D-dimer, a noninvasive, simple, inexpensive and largely available test, in detecting LAA thrombus in persistent AF patients before planned DC cardioversion.

Methods

Study population

A total of 65 patients (37 men and 28 women; mean age, 56.89±11.03 years) with persistent AF scheduled for DC cardioversion therapy were included in this study. Patients were admitted with complaint of cardiac palpitation continuing for at least one week and AF was diagnosed by transthoracic echocardiography. AF diagnosis and classification was accomplished according to the AHA-ACC and ESC guidelines [3]. Conditions and use of medications that can alter plasma D-dimer independently of AF were excluded. Exclusion criteria were: recent anticoagulant use, presence of a prosthetic valve, deep vein thrombosis, acute and chronic infection, malignancy, inflammatory diseases, recurrent pulmonary embolism, acute or chronic aorta dissection, aorta aneurysm, pregnancy, smoking, acute coronary syndrome, congestive heart failure, chronic renal failure, and acute stroke. Patients were informed regarding the study and written consent was obtained. Study was approved by the hospital's internal review board.

Echocardiographic analysis

Before TEE, all patients were evaluated regarding the presence of valve disease, ejection fraction rate and left atrium dimensions using transthoracic echocardiography (Vivid 7 digital ultrasound equipment, Vingmed, GE) with a multifrequency transducer. Standard TEE was performed in all patients using TEE Vivid digital ultrasound equipment (Vingmed, GE), with a 5 MHz transducer. Left atrial appendage thrombus was diagnosed based on presence of three of the below criteria: mass with defined borders, distinct echogenicity differing from the underlying tissue, independent mobility, and detection in a few different imaging planes.

Blood analysis

Quantitative analysis of plasma D-dimer was performed using MDA Auto-dimer (Trinity Biotech, USA) immunoturbidimetric assay. Plasma D-dimer levels were measured by a physician blinded to the study. Blood samples were drawn following 12-hour fasting from antecubital vein and were analyzed within 2 hours.

Statistical analysis

Statistical analysis was performed using NCSS (Number Cruster Statistical System) 2007&PASS 2008 Statistical Software (Utah, USA). Data were expressed using descriptive statistics (mean, standard deviation, frequency). Qualitative data with normal distribution were compared between groups using Mann Whitney U test. Quantitative data were compared using Chi-square, Fisher's Exact Chi- square or Mc Nemar test as appropriate. D-dimer cut-off value was established through Receiver Operating Curve (ROC) analysis (Medcalc statistics program). Logistic analysis was performed as multivariate analysis. Results were evaluated within 95% confidence interval (CI), at $p<0.05$ level of significance.

Results

TEE examination revealed LAA thrombus in 19 of 65 patients. Clinical characteristics of patients with and without thrombus are presented in Table 1. Hypertension and mitral stenosis were significantly associated with LAA thrombus ($p<0.05$), while other risk factors such as age, diabetes, low ejection fraction (<50%) and mitral regurgitation were not (Table 1).

Serum D-dimer levels of patients ranged between 0.025 and 25.80 μg/ml, with 3.91 ±4.97 μg/ml mean, and 2.25 μg/ml median. ROC analysis was performed and optimal D-dimer cut-off value

Table 1. Risk factors in patients with or without LAA thrombus.

		LAA thrombus n=19	No thrombus n=46	*p*
Age (mean±SD)		56.94±9.14	56.86±11.81	*0.980**
Hypertension, n(%)		16 (84.2)	23 (50.0)	*0.010†*
Diabetes mellitus, n(%)		3 (15.8)	10 (21.7)	*0.585†*
Ejection fraction <50%, n(%)		5 (16.3)	7 (15.2)	*0.294‡*
Mitral stenosis	None	10 (52.6)	40 (87.0)	*0.003‡*
	Mild	0 (0.0)	1 (2.2)	
	Moderate	5 (26.3)	5 (10.9)	
	Advanced	4 (21.1)	0 (0.0)	
Mitral regurgitation	None	18 (94.7)	37 (80.4)	*0.356‡*
	Mild	0 (0.0)	6 (13.0)	
	Moderate	1 (5.3)	2 (4.3)	
	Advanced	0 (0.0)	1 (2.2)	

** Student t test; † Mann Whitney U test; ‡ Chi-square test*

Table 2. Plasma D-dimer levels in patients with and without LAA thrombus

Plasma D-dimer	LAA thrombus n (%)	No thrombus n (%)	Total	p
High (>1.60 µg/ml)	18 (27.7)	10 (15.4)	28 (43.1)	0.0117
Low (≤1.60 µg/ml)	1 (1.5)	36 (55.4)	37 (56.9)	
Total	19 (29.2)	46 (70.8)	65 (100)	

Mc Nemar Test

was established as 1.60 µg/ml (Figure 1). Area under the curve (AUC) was determined as 0.863 (%95 CI, 0.755-0.935). At 1.60 µg/ml cut-off, D-dimer test performed with 95% sensitivity (95% CI, 74.0-99.9), 78% specificity (95% CI, 63.6-89.1), 64% positive predictive value, 97% negative predictive value, 4.36 positive likelihood ratio and 0.067 negative likelihood ratio.

In Mc Nemar test LAA thrombus and plasma D-dimer levels were significantly associated (p=0.0117, Table 2). Only one patient with low plasma D-dimer (<1.60 µg/ml) had LAA thrombus on TEE.

Backward stepwise logistic regression analysis with plasma D-dimer, hypertension and mitral stenosis variables yielded a statistically significant model (p<0.001) with Negelkerke R square value of 0.846.

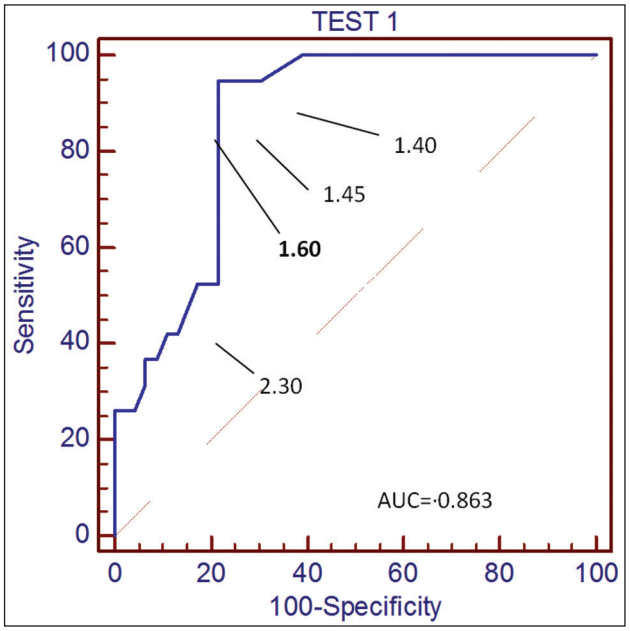

Figure 1. Receiver operating characteristic (ROC) curve determined the optimal D-dimer cut-off value as 1.60 µg/ml. Area under the curve (AUC) was determined as 0.863 (%95 CI, 0.755-0.935).

Discussion

In this study we included younger (mean age 57±11), non-anticoagulated, and persistent AF patients without CHF or recent thromboembolic event. Our objective was to determine if we could use a noninvasive marker in this patient population to stratify thrombus risk and spare low-risk patients the invasive TEE procedure. Our study shows that serum D-dimer test can be used to exclude LAA thrombus in persistent AF patients scheduled for DC cardioversion. Serum D-dimer levels measured by a quantitative immunoturbidimetric assay excluded LAA thrombus with 95% sensitivity and 97% NPV at 1.60 μg/ml cut-off established by the ROC analysis.

D-dimer is a fibrin degradation end-product and at elevated levels suggests active thrombogenesis/fibrinolysis process within the cardiovascular system. D-dimer is a sensitive test with a high NPV, but it is a non-specific marker which can increase in response to a variety of other nonthrombotic conditions such as surgery, inflammation, pregnancy or cancer. Therefore, serum D-dimer is more reliable as an exclusion marker rather than a diagnostic marker [9]. Currently, serum D-dimer is used in exclusion of venous thromboembolic diseases such as deep vein thrombosis and pulmonary embolism in moderate/low risk patients. Previously, two studies investigated the potential role of serum D-dimer levels for predicting LAA thrombus in patients with AF. In their study including 73 patients presenting with AF lasting more than 48 hours or atrial flutter with documented history of AF, Somloi et al., showed that using a serum D-dimer cut-off value of 0.60 μg/ml LAA thrombus could be excluded with 89% sensitivity and 98% NPV, although specificity (75%) and PPV (33%) were lower [10]. In a more recent study, Habara et al., investigated the serum levels of D-dimer in 925 patients with non-valvular AF, including 250 patients with paroxysmal AF, 84 patients with persistent AF, and 591 patients with permanent AF [11]. Patients with anticoagulant use, CHF and recent thromboembolic event were included in their study. D-dimer cut-off at 1.15 μg/ml had a sensitivity of 76%, specificity of 73%, PPV of 22% and NPV of 97% in their overall population. Higher sensitivity achieved in our study (95%) could be related to our study's confinement to a more homogenous study population with respect to the previous studies. In addition, the quantitative immunoturbidimetric D-dimer test used in our study may have contributed to its higher sensitivity.

In our study hypertension and mitral stenosis emerged as significant risk factors for LAA thrombus formation in persistent AF patients. Mitral stenosis is a rheumatic valvular disease commonly associated with AF, presenting with an increased risk of stroke or thromboembolic event [12, 13]. Interestingly, we observed that patients with mitral stenosis but no LAA thrombus had serum D-dimer levels within the normal range. In a study conducted by Yasaka et al., D-dimer was proposed as an intracardiac mobile thrombus marker for patients with mitral stenosis with or without atrial fibrillation [14].

Hypertension was another risk factor associated with LAA thrombi in our study. Although there may not be a direct cause-and-effect relationship between hypertension and thrombus formation, hypertension is an important predictor of stroke, and is present in all scoring systems developed for stroke-risk stratification in AF, such as CHADS(2), CHA(2)DS(2)VASc or van Walraven [15]. However, in a recent case-control study including non-anticoagulated nonvalvular AF patients with (n=110) and without (n=387) LAA thrombus, hypertension was not a significant independent predictor of LAA thrombus [16]. Instead, CHF, prior stroke/transient ischemic attack, diabetes, permanent AF, AF duration and spontaneous echocardiographic contrast (SEC) were determined as independent predictors of LAA thrombus [16]. Similarly, Habara et al., also found CHF and previous stroke as independent predictors of LAA thrombus [11]. These two variables did not emerge in our analysis since both conditions can increase D-dimer levels independently of LAA thrombus and thus were among the exclusion criteria for the study.

Small population size is a limitation of this study. Larger multicenter studies may allow development of a clinical scoring system with serum D-dimer as one of the parameters to establish the risk of LAA thrombus in patients with persistent AF.

In conclusion, quantitative serum D-dimer measurement is a sensitive test with a high NPV to exclude LAA thrombus in persistent AF pa-

tients before planned DC cardioversion therapy. D-dimer test may be used in qualified patients to avoid invasive TEE procedures and unnecessary anticoagulation before cardioversion.

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Endobronchial tuberculosis: Clinical and bronchoscopic features

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Abstract

The aim: To determine the clinical and bronchoscopic features of EBTB in Serbia in eight-year period.

Methods: Retrospective clinical study was conducted at the Institute for Lung Diseases and Tuberculosis, Clinical Center of Serbia. In the period from 01/02/1999. to 01/12/2007. all cases of patients with histologically verified endobronchial tuberculosis were analyzed. For every patient we had a questionnaire filled out with medical history, physical exam, chest radiographs, bronchoscopic examination with sample collection (biopsy and aspirate). Statistical differences were evaluated using the chi-square test and *t*-test.

Results: In 88 patients, bronchoscopic biopsy confirmed the diagnosis of EBTB. There were more men (58.0%) than women. Most patients (96.6%) had symptoms before diagnosis; cough and fatigue were most dominant (75% and 53%). The duration of cough varied from 1 to 450 days, but in only 7 (10.6%) patients cough lasted longer than 6 months. For almost half of patients fatigue occurred later than 3 months before the diagnosis. In more than half of patients who had a fever, it lasted for up to a month. In a quarter of people who have had weight loss, it lasted longer than 3 months. Hemoptysis had only 9 (10.9%) patients. In most cases endoscopic findings were not specific for tuberculosis (edematous-hyperemic, and chronic-bronchitic forms). In only 11.4% of patients who had active caseating EBTB, bronchoscopic finding indicated on tuberculosis. In 5.7% of patients tumorous form EBTB was seen, while fibrostenotic (late) forms were present in less than 7% of patients.

Conclusion: In our population, which consisted mostly of patients less than 50 years of age, single most frequent form was nonspecific bronchitic one from which more frequently suffered younger patients, with excellent prognosis after antituberculous treatment and complete sanation of lesions, without consequences. The most complicated form, fibrostenotic, was rare in our population. Also, in patients who reported earlier to the doctor, because of cough, edematous hyperemic form was significantly more common with good prognosis if treatment is started earlier. Most of the patients in our population were of young and middle age, which only points out the need to early recognize, diagnose and treat EBTB.

Key words: EBTB, clinical features, endoscopic types.

Introduction

EBTB is chronic, progressive tuberculous infection of bronchi and/or trachea which is characterized with infiltration of mucosa with: hyperemia, swelling, granulomatous tissue formation, occasionally ulcerations and finally, bronchostenosis, as a result of scarring formation process . EBTB is present in 10-40% of patients with active tuberculosis and in more than 90% it has complicated clinical course, with bronchostenosis formation. Its incidence tends to decline with advancement of antituberculous treatment, but it remains to be the disease which occurs mainly in people younger than 35 years of age. Endobroncial tuberculosis may start gradually, mimicking lung cancer or rapidly, which can mimic asthma, aspiration pneumonia, or foreign body aspiration [1]. EBTB symptoms may occur even after completion of antituberculous therapy. This form of TB is most common in people of younger age, with a slight dominance of disease in women [2] [3] [4]. About 15% of elderly patients may also have EBTB [5]. The predominant symptom in most patients is barking cough which does not respond to antitusic medications [6] [7]. Hemoptysis are common, but massive hemoptysis rarely appear.

Radiographic findings in 10% to 20% of patients with EBTB be described as normal, so normal radiographic findings do not exclude the diagnosis of EBTB [8]. Radiographic manifestations of tuberculosis include persistent bronchostenosis, lobar or segmental collapse, hyperinflation and postobstructive lobar pneumonia. Computed tomography (CT) of the chest allows precise localization and assessment of involvement of the peribronchial structures. Typical HRCT (high resolution computed tomography) findings in the EBTB are centrilobular asymmetric nodules and branching lines with unilateral or bilateral distribution[8] . This phenomenon is known in literature as "tree-in-bud".

Standard for tuberculosis diagnosis is microbiological confirmation on Lowenstein-Jansenn medium but bronchoscopy with bronchial mucosal biopsy is essential in the diagnosis of EBTB, because the endoscopic findings are positive in 90% patients [3][10] [11] [12][13]. Histologic confirmation of EBTB requires that bronhoscopist has to choose the best place and most appropriate instruments to make biopsy that will provide high quality tissue samples.

Endoscopic appearance of lesions has also prognostic value. Chung and Lee in 2000 [2] proposed the following bronchoscopic classification of EBTB: 1. Active caseating form 2. Edematous-hyperemic form 3.Fibrostenotic form 4. Granular form 5. Tumorous form 6. Ulcerative form 7. Nonspecific bronchitic form. Granular, ulcerative and non specific bronchitic form tend to heal without consequences while other subtypes, especially fibrostenotic, have significantly poorer prognosis. Early diagnosis, where bronchoscopy has an indispensable role, and early initiation of treatment can significantly change the unfavorable natural course of untreated EBTB. Bronchoscopy is essential not only in diagnosis but has an important role in treatment planning and therapy. In some cases, when irreversible changes occur interventional bronchoscopic methods can be used as alternative or preparation for surgery

The aim is to focus attention on this rare form of TB, recognize it as soon as possible and refer the patient to the bronchoscopy, to obtain the diagnosis and initiate the treatment which prevents frequent complications that are specific to EBTB, especially regarding the fact in majority of these patients, microbiological confirmation is not achieved in the first few months of disease.

Methods

Retrospective clinical study was conducted at the Institute for Lung Diseases and Tuberculosis, Clinical Center of Serbia in the period from 01/02/1999. to 01/12/2007. In 88 patients, bronchoscopic biopsy confirmed the diagnosis of EBTB. Indication for bronchoscopy in these cases was suspicion on other lung diseases, mainly bronchogenic carcinoma. All these patients (88) were analyzed.

Subject selection and data collection

In each patient with EBTB the following was done:

- Medical history
- Clinical examination
- Chest radiography
- Bronchoscopy with biopsy

Questionnaire was filled for each patient which contained the following elements:

- Demographic characteristics of patients
- The medical history:
  - Earlier treatment of tuberculosis and tuberculosis localization
  - Clinical findings: the presence of symptoms and length of their duration at the time of diagnosis (cough, hemoptysis, fever, malaise, weight loss, etc.). Endoscopic findings were described using above mentioned Chung and Lee classification. All questionnaires were coded, data on the characteristics coded, a database was created and computer processing performed by appropriate statistical techniques.

Analysis and data processing consisted of two parts. The first part consisted of descriptions of all hospitalized patients. In the second part, we analyzed the statistical association of individual variables: clinical and endoscopic features of EBTB. Statistical significance of variables was assessed by $\chi 2$ test and t test.

Results

Between 01 02. 1999. and 31.03.2007. there has been 29,605 fiber optic bronchoscopies in the Institute for Lung Diseases and Tuberculosis, Clinical Center of Serbia. In 88 (0.29%) patients, bronchoscopic biopsy confirmed the diagnosis of EBTB. There were more men (58.0%) than women (42.0%). The sex ratio was 1.38 for men. The youngest patient at the time of diagnosis was 16 years old and the oldest 73 years (X=44.7, SD=16.2).Women with EBTB were significantly younger (under 40 years) than men (χ^2=11.1 df=5 p=0.05). Most male patients were in the age group of 40 to 60 years. Previously treated tuberculosis had only one patient.

Symptoms and duration of symptoms

Most patients (85-96.6%) had symptoms before diagnosis, whereas 3 patients (3.4%) were asymptomatic. Apart from three patients, all the rest had at least one symptom of tuberculosis [Table 1]. Cough and fatigue were most dominant symptoms in our patients. The duration of cough was from 1 to 450 days, but in only 7 (10.6%) patients cough lasted longer than 6 months. Fatigue is the longest-lasting: 450 days, but for almost half of patients (48) fatigue occurred later than three months before the diagnosis of EBTB. In more than half (55.5%) patients who had a fever, fever lasted for up to a month. In a quarter of people who have had weight loss, it lasted longer than 3 months. Hemoptysis had only nine (10.9%) patients, whereas in 3 of them haemoptysis lasted longer than two weeks.

There was no statistically significant differences in any of the clinical symptoms in relation to age of patients: cough (χ^2=5.1 DF=5 p=0.4), hemoptysis (χ^2=3.0, df=5 p=0.7), fever (χ^2=8.7 DF=5 p=0.1), fatigue (χ^2=5.6 DF=5 p=0.3) and weight loss (χ^2=2.4 DF=5 p=0.8). The average duration of symptoms to diagnosis EBTB in relation to gender and age of patients is shown in Table 2 [Table 2].

Except of the duration of cough, all other symptoms (hemoptysis, fever, fatigue and weight loss) lasted longer in males, but the difference was not statistically significant.

Endoscopic findings

Endoscopic finding in our patients are described in Table 3 [Table 3].

Table 3. Endoscopic finding in patients with EBTB

Form of EBTB	Number of PTS (%)
Active caseating	10 (11,4)
Edematous hyperemic	29 (33,0)
Fibrostenotic	6 (6,8%)
Tumorous	5 (5,7%)
Granulation	2 (2,3%)
Ulcerous	0 (0,0)
Nonspecific bronchitis	35 (39,8)
Σ	88 (100,0)

Table 1. The number and percentage of patients with symptoms and duration of symptoms

Symptoms	Number of pts (%)	Duration of symptoms to diagnosis (days)		
		Average (sd)	Median	Min-max.
Cough	66 (75,0)	97,4 (95,7)	60,0	1- 450
Hemoptisis	9 (10,2)	52 (96.9)	10,0	1- 300
Febrility	40 (45,5)	51,1 (37,3)	30,0	2 -150
Fatigue	47 (53,4)	92,4 (94,8)	60,0	10 - 450
Loss of body weight	36 (40,9)	97,8 (100,9)	60,0	21 - 450

Table 2. The duration of symptoms to diagnosis EBTB in relation to sex and age

Average duration of symptoms	Sex X (SD)		*t*- test	DF	*p*
	Male	Female			
Cough	92,1 (95,6)	107,4(96,9)	0,721	64	0,47
Haemoptisis	57,2 (102,2)	10,0	0,436	7	0,68
Febrility	51,3 (37,8)	50,9 (37,6)	0,028	38	0,98
Fatigue	107,5 (112,3)	65,6 (42,1)	1,48	45	0,15
Loss of body weight	114,8 (120,7)	67,7 (38,3)	1,36	34	0,18

In most cases (64) endoscopic findings were not specific for tuberculosis, because of the prevailing edematous-hyperemic (29), and chronic-bronchitic forms (35). In only 11.4% of patients (who had active caseating EBTB) bronchoscopic findings indicated the possibility of tuberculosis. In 5.7% of patients tumorous form EBTB was seen, while fibrostenotic (late) forms were present in less than 7% of patients.

Bronchoscopic findings in relation to gender of patients are shown in Table 4. [Table 4]

There was no statistically significant difference in bronchoscopic findings in relation to gender of patients with EBTB ($\chi^2 = 3.8$ DF = 5 p = 0.6).

If the bronchoscopic findings are observed separately, it is evident that the active caseating form of EBTB is more often found in older persons [Table 5] Also, nonspecific bronchitis form was significantly more frequently found in younger patients [Table 6]

Edematous hyperemic form was significantly more found if the patients addressed to the doctor in a shorter time interval from the onset of cough [Table 7], while in relation to the duration of other clinical symptoms and edematous hyperemic finding no statistically significant difference was found. Bronchoscopic finding of nonspecific bronchitis was not associated with the duration of any clinical symptoms: cough duration (t = 0.667 DF = 64 p = 0.5), hemoptysis duration (t = 0.463 df = 7 p = 0.6), duration of fever (t = 0.847 DF = 38 p = 0.4), duration of fatigue (t = 0.785 DF = 45 p = 0.4), duration of loss of body weight (t = 0.480 DF = 34 p = 0.6) Bronchoscopic findings in other forms of EBTB was not associated with the duration of symptoms.

Table 4. Bronchoscopic findings in endobronchial tuberculosis in relation to gender of the patients

Bronchoscopic finding	Sex Number (%)		Total
	Male	Female	
Active caseating	5 (9,8)	5 (13,5)	10 (11,4)
Edematous hyperemic	18 (35,3)	11 (29,7)	29 (33,0)
Fibrostenotic	4 (7,8)	2 (5,4)	6 (6,8%)
Tumorous	1 (2,0)	4 (10,8)	5 (5,7%)
Granulation	1 (2,0)	1 (2,7)	2 (2,3%)
Ulcerous	0 (0,0)	0 (0,0)	0 (0,0)
Nonspecific bronchitis	22 (43,1)	14 (37,8)	35 (39,8)
Σ	51 (57,9)	37 (42,1)	88 (100,0)

Table 5. Active caseating EBTB in relation to the age of patients

Active caseating form	Number of patients	Age	
		Average value (x)	Standard Deviation (SD)
No	78	42,4	15,99
Yes	10	53,5	15,38

t= - 2,07 DF=86 p<0,41

Table 6. Nonspecific bronchitis form of EBTB in relation to the age of patients

Nonspecific bronchitis EBTB	Number of patients	Age	
		Average value (x)	Standard Deviation (SD)
No	52	47,1	16,35
Yes	36	38,7	14,89

t= 2,471 DF=86 p=0,01

Table 7. Bronchoscopic findings in relation to duration of cough

Edematous hyperemic form	Number of patients	Age	
		Average value (X)	Standard Deviation (SD)
No	45	115,6	108,39
Yes	21	58.4	40,21

t= 2,34 DF=64 p<0,02

Discussion

In our study, 88 patients with EBTB were analyzed in 8-year period and in majority of them, microbiological confirmation of tuberculosis was not achieved by sputum examination and therefore they were referred to bronchoscopy, on suspicion on other lung diseases, bronchogenic carcinoma in particular. The significance of early diagnosis and recognition of these rare form of TB points out the result in our study that majority of the patients (53-60.2%) in our study were of younger age (16-50years). These results coincide with the findings of other authors [2][18] but women were significantly younger age (40 years) than men. Similar observations have Song et al [19] and Shim et al [20]. In this study, men were more likely than women affected with the EBTB (the ratio was 1.38 for men) which is in contrast with the results of Lee et al. [3] Daly et al. [14] and Chung et al [2], and other authors[4], [15] [16] mostly from Korea and Japan. These differences may be explained by factors such as racial and genetic predisposition.

The analysis of the most frequent symptoms and their duration can significantly earlier refer the patient to the bronchoscopy, despite the fact that these symptoms are non specific for EBTB and can be present in other lung diseases. Most patients (96.6%) in this study had one or more analyzed symptoms before diagnosis, while without symptoms were only 3.4% of patients, similar as in a study of Hoheisel et al. [9] , but very different from the study of Lee et al [3] (24%). The most common symptom was cough (75% of patients) which coincides with the experience of other authors[6] [20]. Intersting fact is that fever followed the course of EBTB in 45% of our patients, which can be found also in the studies of Hoheisel et al [9] , Lee et al. [3] while at van der Brande et al [5] was recorded in up to to 87% of cases. It can be partly explained by the fact that sub-febrile temperature in the afternoon and night time, characteristic for the tuberculosis, go unnoticed and that most patients in our study did not not measure body temperature when feeling unwell.

Haemoptysis in this study been seen in 10.2% patients, while in other the frequency ranged from 1.2%[20] up to 25% [6]. Endobronchial localization of tuberculous process is not associated with a high incidence of hemoptysis but their occurrence is dramatic to the patient and it leads to urgent bronchoscopy and diagnosis of EBTB. The symptoms in most patients are cough and fatigue, while the lost time to diagnosis is longer in those patients whose clinical picture is dominated by weight loss and cough. Hemoptysis and fever lead patient earlier to the doctor and the diagnosis. In support of these findings is the fact that the average time to diagnosis in the studies of other authors [6][7][18] [22] was from 11 to 26 weeks (up to one year), while the results of this study showed the average duration of symptoms of 11.2 weeks, but, in a significant number of patients, with a maximum period of more than one year (64 weeks).

Endobronchial form of TB can only be diagnosed by bronchoscopy

The results of this study are most similar to the study of Morrone et al [23] who found in 75 examined patients the most-represented non-specific bronchitic form (48% vs 39.8% of patients in this study), edematous-hyperemic (in 28.6% vs 33% of our patients), while other forms were rare (fibrostenotic 9% of patients vs our 6.8%) and active-caseating form of EBTB was not seen in any of the patients (in our study, this form was found in the 11.4 % of patients), Kim et al [24] so-called exudative changes (edematous-hyperemic and non-specific bronchitic type) found in 43.3% of 162 patients. Chung and Lee [2], on the contrary, found only 7.9% of patients with non specific bronchitic form, while edematous-hyperemic was present in 14% of their patients. The obvious difference in the data is probably related to the duration of disease; this study proved that the edematous-hyperemic type of disease was more common in those who were earlier came to the doctor because of cough. Active-caseating form is more frequently found in older people (there are no data for comparison from the literature) while the non-specific bronchitis was significantly more common in younger patients.

Conclusions

In our population, which consisted mostly of patients less than 50 years of age, single most frequent form was nonspecific bronchitic one from which more frequently suffered younger patients,

with excellent prognosis after antituberculous treatment and complete sanation of lesions, without consequences. The most complicated form, fibrostenotic, was rare in our population. Also, in patients who reported earlier to the doctor, because of cough, edematous hyperemic form was significantly more common with good prognosis if treatment is started earlier. Most of the patients in our population were of young and middle age, which only points out the need to early recognize, diagnose and treat EBTB.

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An investigation of atherosclerotic markers in patients with subjective tinnitus

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Abstract

Objective : Aim of this study is to investigate the presence and magnitude of atherosclerosis by non-invasive methods in carotid and femoral arteries of patients with subjective tinnitus.

Methods: Cases were admitted to ENT clinics with a complaint of tinnitus. A clinical diagnosis of subjective tinnitus was reached with normal results of autor hinolaryngological examinations. Routine audiologic, biochemical and imaging tests were considered. Data from the control group was obtained by getting permission from the cases referred to the radiology department from other clinics for an ultrasound examination. Intima-media thickness (IMT) and arterial stiffness of carotid and femoral arteries were also measured. Statistical assessment was carried out on high significance levels.

Results: There was no statistically significant difference between the tinnitus group and the control group in terms of carotid and femoral elastic modulus and femoral IMT measurements. Carotid IMT measurements were significantly higher in the tinnitus group in comparison with the control group. Carotid artery cross-sectional compliance and cross-sectional distensibility were significantly lower in the tinnitus group. Femoral artery IMT measurements, cross-sectional compliance and cross-sectional distensibility were significantly lower in the tinnitus group.

Conclusion: Atherosclerosis should be considered as an etiological factor in cases with subjective tinnitus and preventive and / or therapeutic approaches should be planned.

Key words: Tinnitus, arterial stiffness, IMT.

Introduction

Symptoms such as ringing or humming of one or both ears without any stimulus is called "tinnitus". Its prevalent in between 7-32% of population (1, 2). Tinnitus may be classified as "pulsatile-non-pulsatile" or "objective-subjective". Subjective tinnitus is heard by the patient only, whereas objective tinnitus is the ringing or humming sound heard by both the examining physician and the patient. Objective tinnitus is frequently of vascular origin (Dural arterio-venous malformation, carotid-cavernous fistula, arterio-venous malformation of the vascular structures of the neck, etc.). Subjective tinnitus is seen more frequently. Non-pulsatile tinnitus is nearly always subjective (3). One of the frequent causes of non-pulsatile tinnitus is cerebello-pontine junction neoplasies, and the most frequent cause is acustic neurinomas (4). Loud sound, exposure to autotoxic agents, sudden loss of hearing, middle ear or sinus infections, head-neck injuries, arterial hypertension, diabetes mellitus and other metabolic disorders may also cause subjective tinnitus.

It is believed that co-existent atherosclerosis in patients with tinnitus may contribute as an etiological factor by disturbing cochlear microcirculation. This in turn may cause a decrease in blood flow of the inner ear or may cause turbulence in the flow. The association of increased lipid levels with sensorineural hearing loss, subjective tinnitus and vertigo was investigated in a study by Pulec et al. The authors found hyperlipoproteinemia in 5.1% of the cases, with high values in most of the cases and co-existent diabetes mellitus. An improvement in the symptoms were observed after a high-protein, low carbohydrate diet and vasodilator treatments for 5 months (5).

In light of the studies showing an etiologic role played by atherosclerotic risk factors such as diabetes mellitus, hyperlipidemia, and hypertension in tinnitus, non-invasive methods like arterial stiffness

measurements or intima-media thickness measurements may be used to search for atherosclerosis in these patients (6). These investigation methods were shown to be reliable non-invasive tools in showing atherosclerosis in several studies before (7, 8).

This study was to investigate the presence and magnitude of atherosclerosis with non-invasive methods in patients being admitted at the ENT clinics due to tinnitus, with carotid and femoral artery IMT and arterial stiffness measurements.

Material and methods

Ethical approval was obtained from the Ethics Committee of our institution.

Study population

Patients admitted at the ENT clinics during a period of 6 months in 2010 with a complaint of tinnitus. Autorhinolaryngologic examinations and routine audiologic, biochemical and imaging test results that were normal, were also included in this study.

Control group data was obtained from patients being admitted at the department of radiology for routine US imaging. No pathological changes were detected, and with the patients' informed consent.

BMI and waist-hip ratios of all patients were measured. IMT and arterial stiffness measurements were also made.

Analytical methods (ultrasonography)

Carotid and femoral arterial IMT measurements were performed on patients with tinnitus. The measurements were made 2 cm proximal to the bifurcation of right carotid artery and 2 cm distally to the beginning of the deep branch of the right femoral artery. Age, body weight, height, waist and hip measurements and other findings of all persons were also recorded.

All non-invasive measurements were done by the same investigator, using an ultrasound imager (SSA-660A Xario, PLT-704AT probe [Toshiba Medical Systems Corporation, Tochigi, Japan). Intima-media thickness (IMT), lumen diastolic (dD) and systolic (sD) diameters were measured at the common carotid and the femoral artery according to the previously described procedure (9).

The lumen cross-sectional area was calculated as $\pi dD^2/4$ and wall cross-sectional area as $\pi(dD/2+IMT)^2-\pi(dD/2)^2$. Cross sectional compliance and distensibility of the common carotid artery were calculated from diameter changes during systole and from simultaneously measured pulse pressures (ΔP) according to the following formula:

$$\text{Cross sectional compliance}=\pi[(sD^2- dD^2)]/4\ \Delta P$$

$$\text{Cross sectional distensibility}=(sD^2-dD^2) / (dD^2.\ \Delta P)$$

Diastolic wall stress was calculated as using the mean arterial pressure multiplied by dD /21MT. While compliance provides information on the elasticity of the artery as a hollow structure, the incremental elastic modulus provides information on the properties of the wall material independently from the arterial geometry. This variable was calculated as 3 / (1 + lumen cross-sectional area / wall cross-sectional area) divided by cross-sectional distensibility. Repeatability of measurements was assessed as previously described (9).

Statistics

Data is expressed as mean±SD. The differences between data was studied by Student t test and the Mann-Whitney U Test. The level of statistical significance was determined at $p<0.05$. The data was analysed using SPSS for Windows v. 15.0 (SPSS Inc., Michigan, IL, USA).

Results

The mean age of the study group is 86. Tinnitus (55 males and 31 females) and 129 in the control group (73 males and 56 females) is 48±19 (Table 1).

No statistically significant differences were detected between the control and the tinnitus groups in terms of age ($p=0.815$), BMI ($p=0.737$) and WHR ($p=0.264$).

No statistically significant differences were detected between the control and tinnitus groups in terms of carotid and femoral elastic modulus and femoral IMT measurements.

Carotid artery IMT measurements of the tinnitus group were statistically significantly higher than the control group ($p<0.05$) (Table 2).

Carotid artery cross-sectional compliance and cross-sectional distensibility of the tinnitus group were significantly lower than that of the group control ($p<0.001$) (Table 2).

Table 1. Ages, BMI, WHR values of the investigated groups

	Controls		Tinnitus		p value
	Mean	Std. Deviation	Mean	Std. Deviation	
Age	48.93	19.65	49.52	15.44	0.815
Body Mass Index (kg/m2)	28.55	5.38	28.8	5.3	0.737
Waist/Hip Ratio	0.82	0.06	0.83	0.07	0.264

Table 2. Carotid and femoral artery IMT and arterial stiffness measures of investigated groups.

	Controls		Tinnitus		p value
	Mean	Std. Deviation	Mean	Std. Deviation	
Carotid IMT (mm)	0.39	0.13	0.43	0.15	0.039
Carotid Cross-sectional compliance	0.201	0.006	0.176	0.006	0.000
Carotid Cross-sectional distensibility	0.0082	0.0003	0.0069	0.0003	0.000
Femoral IMT (mm)	0.39	0.15	0.33	0.13	0.003
Femoral Cross-sectional compliance	0.18	0.01	0.13	0.004	0.000
Femoral Cross-sectional distensibility	0.0079	0.0004	0.0061	0.0002	0.000

Femoral artery IMT measurements of the tinnitus group were statistically significantly lower than the control group ($p<0.01$) (Table 2). Femoral artery cross-sectional compliance and cross-sectional distensibility of the tinnitus group were significantly lower than the control group ($p<0.001$) (Table 2).

Discussion

Tinnitus is a frequently encountered health problem seen especially in an over 40 year old person. It is especially in common industrialized countries (10). Tinnitus may be classified as pulsatile-non-pulsatile or as objective-subjective. Hypertension and metabolic diseases such as diabetes mellitus may play a role in the etiology of tinnitus (11). However, the exact etiologic mechanism of tinnitus is still not completely understood (12).

Arterial stiffness reflects the mechanical stress and elasticity of the arterial walls. An increase in aortic stiffness values were shown in association with the presence of hypertension, diabetes mellitus, smoking and normal aging (13). Many parameters were developed as markers of arterial stiffness and it is known that no one marker is superior to another. The functional changes that occur in the early atherosclerosis phase, (when generally there are no clinical findings) were mainly investigated in the aorta, brachial and femoral arteries (14). Aortic stiffness measurements of the carotid arteries were done in ARIC and SMART studies, they were found to be another risk factor for atherosclerosis (15-17). Also, IMT measurement of the carotid artery was found to be a reliable, non-invasive marker to show the presence and magnitude of atherosclerosis in many previous studies.

Glucose metabolism disorders such as diabetes mellitus, hyperinsulinemia were shown to play a role in the etiology of inner ear diseases such as: vertigo, tinnitus, and hearing loss in a study by Kazmierczak et al, but the effect of lipid metabolism disorders could not be conclusively shown (10).

Basut et al have shown that hyperinsulinemia may play a role in the etiology of tinnitus and a diabetic diet in these patients may cause a significant decrease in tinnitus (18).

The presence of obesity and arterial hypertension were searched for in patients with tinnitus or a hearing loss of unknown etiology in a study by Doroszewska et al, investigating the risk factors in inner ear diseases. Patients with an inner ear disorder were found to be heavier than the control group. Systolic and diastolic hypertension were reported to be more prevalent in this group (19).

Carotid and femoral artery IMT measurements and arterial stiffness measurements were done in this study as non-invasive markers of atherosclerosis in patients with tinnitus, and carotid IMT. Measurements were higher in the group with tinnitus, although lower in the femoral artery. Carotid and femoral artery cross- sectional compliance and cross-sectional distensibility measurements as a marker of arterial stiffness were statistically significantly lower in the group with tinnitus.

Conclusion

The results of this study show that atherosclerosis is seen in higher rates in patients with tinnitus, in accordance with other studies done with patients having known risk factors of atherosclerosis such as: hyperlipidemia, obesity, hyperinsulinemia, and diabetes mellitus.

Atherosclerosis should be evaluated as an etiologic factor in these cases, and preventive and / or therapeutic strategies should also be considered.

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Hoarseness - A dominant symptom in early otolaringological diagnosis of lung cancer

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Abstract

Introduction: Hoarseness (dysphonia) means any deviation from normal characteristics, height and intensity of voice. Loss of integrity and function n.laryngeus inferior in clinical practice can be a leading character in the diagnosis of malignant lung tumors, mostly lung cancer.

Objective: The aim of this study was to show the incidence of lung cancer in the etiology of laryngeal paralysis, emphasize the importance of early symptoms as hoarseness and laryngeal paralysis as a sign of advanced malignant process in the chest.

Method: Material for analysis was obtained from 46 patients who were examined in otolaringological practice because of hoarseness as the leading symptoms. All patients were treated as non-invasive diagnostic procedures: medical history, complete otorlaryngologic examination with indirect laryngoscopy, diagnostic by computerized tomography (CT) and magnetic resonance imaging (MRI).

Results: Computed tomography has diagnosed lung cancer in 14 male (58.3%) and 10 (41.3%) female patience. Interval duration of hoarseness in a patient with lung cancer is 1-2 months (33.3%), and other localizations 5-6 months (45.5%). At 22 (91.7%) patients with lung cancer and 19 (86.4%) patients with carcinoma of other sites was presented unilateral paralysis of the lower laryngeal nerve. At detailed process of differentiation and diagnosis of metastasis in 39 (84.78%) patients had magnetic resonance imaging. Cause of the damage to the lower laryngeal nerve in the otholaringological patient group was: 83.87% lung cancer, lung cancer other sites 32.6%, carcinoma of the thyroid gland 23.91% and 8.69% of esophageal cancer.

Conclusion: Hoarseness was the dominant symptom and laryngeal paralysis was a dominant sign of grown malignant lesion process followed by a n.laryngeus inferior. Thorough medical history and indirect laryngoscopy in the otolaryngological examination in correlation with modern imaging methods (computed tomography and magnetic resonance imaging) will allow an early diagnosis of bronchial carcinoma.

Key words: Early diagnosis, lung ca, paralysis n. laryngeus inferior, the indirect laryngoscopy, computerized tomography, magnetic resonance imaging.

Introduction

Larynx, as one of the most perfect organ with it's highly specialized functions not only allows breathing but also to expresses consciousness, emotion, reason, intellectuality through speech, man's whole personality.

Hoarseness or dysphonia means any deviation from normal characteristics, height and intensity of voice. Essential relationship between these two entities is the lower laryngeal nerve (sn laringeal inferior recurrent), which innervates internal muskulature of larynx, and whose loss of integrity and function in clinical practice may be the first and main symptom in the diagnosis of malignant lung tumors, mostly lung cancer.

According to the presentation of results unilateral paresis and vocal cord paralysis are frequently encountered in otholaringological practice and usually are the result of n. recurrent's damage. Etiological factor is usually extralaringeal malignancy, iatrogenic or idiopathic cause. Unilateral loss of innervation unables the vocal cords (paramedial position) and causes muscles atrophy. As a result, we have dysphonia, and in several cases aspirations of the position of the immobile vocal cord in cases of mutual paralysis [1].

Monitoring of hoarseness by ENT, especially those who do not respond to therapeutic treatment according to the protocol of diagnostic must awa-

ke suspicion of oncological aspect of diseases. Hoarseness at patients with the lower laryngeal nerve damage, claims in the shortest time possible additional diagnostic using complex methods (computerized tomography - CT, magnetic resonance imaging - MRI) in order to recognize the natural course of the disease at an early stage for timely and optimal treatment.

Specific central ipsi and contralateral and ipsilateral peripheral innervation of the larynx are the reasons why only peripherally vagal lesions influence on the motility of the larynx. Peripheral lesions of n.lararyngeus inferior is usually manifested by ipsilateral vocal cord paralysis.

The entire internal laryngeal musculature, except m. cricothyreoideus, innervated by the motor fibers of the n. laryngeus inferior, while his sensitive branches innervated subglottic mucosa. M. cricothyreoideus only receives motor fibers from the n. laryngeus superior, and all the rest of larynx receives sensory innervation from the lower laryngeal nerve. Both of these nerves are branches of n. vagus, of which are separated in the neck-chest area, asymmetric at different altitudes: right at the height of the IITh (VIIc - IITh vertebra), and left lower, in height of the VTH (IIITh - VIIITh vertebra). Specific branches of the lower laryngeal nerve, the existence of variations and anastomoses provide an explanation why the injuries of the lower branch occurs aphonia (adductor paralysis), in violation of the last branches of the respiratory symptoms (paralysis of the larynx abduktor), and the injury of the nerve stem to a complete paralysis of laryngeal muscles (abduktor, adductor and constrictor). Vulnerability, the return flow and the existence of variation and lower laryngeal nerve anastomosis are often the cause of his lesion in numerous pathological conditions as well as thoracic surgery, thyroid and neck.

In the pathology of the lower laryngeal nerve as often presented etiological factor stands out lung cancer localized in the upper and middle lung parties. Head and neck carcinomas constitute approximately 5% of all malignancies and their frequency is growing worldwide [2]. Expansive growth of certain types of tumors and the rapid creation of regional metastasis in lymph nodes of aortic-pulmonary window, lead to infiltration and compression of recurrent nerve tree which results in complete unilateral paralysis of the muscles of the larynx. Different histopathological groups of lung cancer are very important for understanding the problems of neurogenic paralysis of the larynx, because their appearance is often an indicator of inoperability or cancer because of the affection of vital organs (truncus art. pulmonic, aorta, esophagus, trachea) and metastases in the contralateral mediastinal lymph nodes.

Sophisticated diagnostic imaging methods (spiral CT, MRI) enable early detection of tumors and provide guidance in further management of patients (surgery, chemotherapy, radiotherapy) [2, 3].

Paralysis n. recurrent frequently encountered in everyday otrolaringological practice and neglection of hoarseness with intermittent improving timbre as the leading symptom is often the cause of delayed diagnosis of lung carcinoma. Thorough anamnesis and knowledge of the triad of symptoms characteristic for unilateral vocal cord paralysis should not belong only to experienced ENT, but also by pulmonologists, endocrinologists and above all a general medical practice. Triad of symptoms are: 1 characteristic sudden dysphonia (fonestenic and pneumofonic voice - Ischikawa classification), 2 rapid vocal fatigue (which increases in the evening) and 3 the impossibility of singing. Detailed anamnesis often find out the symptoms that the patient does not pay attention to run a basic problem - suddenly emerged hoarseness or hoarseness that does not go with therapy prescribed: Dysphagia (first solid, then liquid food), shortness of breath due to heavier vocal fatigue, a burning sensation in the throat (due to accompanying inflammation, or sensory neuritis), moderate respiratory failure during physical activity, coughing as an attempt to cleanse the throat or pharynx, aspiration of saliva, food and frequently loss of body weight. Important anamnestic data are the first suspected etiology, and properly performed indirect laryngoscopy by the ENT are important to shorten the diagnostic time.

In routine clinical practice, we note by indirect laryngoscopy that an isolated paralysis n.laryngeus inferior are manifested in typical paramedial position fixed vocal cords, while in bilateral paralysis position is intermediate. Additional radiological, non-invasive diagnostic procedures in the further diagnosis are: standard lung tomography, Rt chest, passage of the esophagus, lung CT (diagnosis of

solitary pulmonary nodules smaller than 5 mm, mediastinal lymph nodes greater than 1 cm), MRI, with a view to reviewing the infiltration of large blood vessels of the mediastinum, heart, spine, neck base, to differentiate soft tissue tumors of hilar and mediastinal masses and for screening of distant metastases primarily in the liver, brain and skeleton.

Of invasive diagnostic procedures, most frequent and invaluable for diagnosis bronchopulmonary disease is rigid, fiber optic and video based bronchoscopy which with set of instruments for taking a biopsy provides material for cytological, histopathological, bacteriological, immunological and histochemical study [3].

Objective

The main symptom - hoarseness and a leading character - a unilateral vocal cord paresis may not be ignored in otolaryngological clinical practice but must be a guideline for differential diagnosis of malignancy. The first step in diagnosis is undoubtedly part of the old otolaryngological method of indirect laryngoscopy which will with organized teamwork of other specialties and application of specialized diagnostic methods detailed measure progress of oncological diseases and impact on treatment planning.

With that in mind, the aim of this study was to show the incidence of lung cancer in the etiology of laryngeal paralysis in otolaringological practice and to emphasize the importance of hoarseness as an early symptom and laryngeal paralysis as a sign of grown malignancy in the chest, when first diagnosed by otolaryngologist, in correlation with the findings of computerized tomography (CT) and magnetic resonance imaging (MRI).

Method

This study included 46 patients with hearing n. laryngeus-and inferior-a. All patients were treated as non-invasive diagnostic methods such as: medical history, complete otolaryngological examination with routine indirect laryngoscopy, CT and MRI diagnostics.

Data important to take from anamnesis are: positive family anamnesis of malignant disease, smoking experience, hoarseness with vocal fatigue, dry cough, thoracic pain, weight loss, shortness of breath, coughing up blood, swallowing disorder, periods of high temperatures.

Indirect laryngoscopy identified the presence of paresis and paralysis of the n. laryngeus inferior.

Computerized tomography (CT) is a radiological diagnostic imaging method that as soon as possible is a computer reconstruction of the cross or axial tomographic layer, on the basis of multiple measurements of the absorption values of X-rays, in our study was conducted on the device TOCHIBA (Japan).

Diagnostic magnetic resonance imaging (MRI) was performed on the device MPR 3 (Hitachi Open System, Japan).

Magnetic resonance imaging (MRI) is the latest radiological diagnostic method of direct representation, which is not based on the "X" air obtaining images (MRI-Magnetic Resonance Imaging). MRI is a noninvasive method of choice in the diagnosis of tumors of the larynx, lungs, mediastinum, esophagus, and tireoidee. We used the standard sequence, multiplanarne plane section thickness of 5 mm.

Paramagnetic contrast in the diagnosis is always used in suspected tumors that gain the signal intensity. The MRI examinations use a paramagnetic contrast, when they had exhausted all possibilities sequences and convincing diagnosis is not set or was not a satisfactory level of representation of pathology eg. The spatial position of pathological changes. In the diagnostics we use contrast Gd-DTPA, Magnevist factory name (Schering). Gd-DTPA is gadolinium solution, of very specific properties. MRI is of particular importance for the interpretation of the relationship of malignant tumors with the structures of aortic-pulmonary window and the large vessels of the mediastinum and its findings clearly determine the selection of therapeutic approach.

Patients with neurological disorders of the larynx were examined in specialised otolaryngological practice "Dr. Dragutinovic" and radiological processed at the Institute of Radiology and Ultrasound "Eurodijagnostika" in Belgrade, during the period of 2006-2010 year.

All patients were reported at otolaryngology examination for the first time because of hoarseness as the leading symptoms.

Results

In the considered four year period (2006-2010 years) 46 patients were examined with paralysis of the larynx, of which 27 (58.7%) were male and 19 (41.3%) were female. Patients on average were 55 years old, men 56 and women 54 and most patients belong to a ten-year age periods. 56-65 years (69.56%), the analysis was not found statistically significant differences by sex and age structure of respondents.

The erliest, leading symptom in all patients was extreme hoarseness which was the main reason of coming to otolaryngologist. Otolaryngologic first rexamination was conducted in 21 patients (45.65%) in whom hoarseness was not therapeutically treated in the previous period, while in 25 patients (54.34%) hoarseness was presented for more than two months without improvements to ordinated treatment.

Hoarseness was treated with the leading therapeutic laryngitis diagnosed by doctors of other specialties in 26 (56.5%) patients, of which lung cancer had 11 (45.8%) patients, while other sites with cancer was 15 (68, 2%) patients.

Interval duration of hoarseness in patients with lung cancer was 1-2 months (33.3%), and other localizations 5-6 months (45.5%). These two intervals of the duration of hoarseness were represented in 30 (75.2%) patients (Table 1).

For detailed case history data were accompanying symptoms which were neglected by patients, all citing the main problem was hoarseness.

Table 1. Ratio of MR-om the findings and duration of hoarseness (in months)

			Duration of hoarseness (in months)				Total
			1-2	3-4	5-6	7+	
MRI	Other localization	Count	7	3	10	2	22
		% within MRI	31.8%	13.6%	45.5%	9.1%	100.0%
	Lung cancer	Count	8	7	5	4	24
		% within MRI	33.3%	29.2%	20.8%	16.7%	100.0%
Total		Count	15	10	15	6	46
		% within MRI	32.6%	21.7%	32.6%	13.0%	100.0%

MRI - magnetic resonance imaging findings;

Table 2. MRI findings correlated with cigarette smoking

			cigarette smoking			Total
			not	moderately	active	
MRI	Other localization	Count	5	6	11	22
		% within MRI	22.7%	27.3%	50.0%	100.0%
	Lung cancer	Count	3	10	11	24
		% within MRI	12.5%	41.7%	45.8%	100.0%
Total		Count	8	16	22	46
		% within MRI	17.4%	34.8%	47.8%	100.0%

MRI - magnetic resonance imaging findings; moderate - to 20; active - over 20

Table 3. MRI findings correlated with the laryngoscopic findings

			Laryngoscopes finding		Total
			paresis	unilateral paralysis	
MRI	other localizations	Count	3	19	22
		% within MRI	13.6%	86.4%	100.0%
	Lung cancer	Count	2	22	24
		% within MRI	8.3%	91.7%	100.0%
Total		Count	5	41	46
		% within MRI	10.9%	89.1%	100.0%

MRI - magnetic resonance imaging findings;

Dry cough was presented at 26 (56.5%) patients, than patients with lung cancer 11 (45.8%), with other sites 15 (68.2%).

Thoracic pain had 14 (30.4%) patients with lung cancer 9 (37.5%), with other sites 5 (22.7%).

Weight loss was observed in 24 patients (52.2%), with the increasing prevalence of cancer in other sites 14 (63.3%) and 10 (41.1%) in patients with lung cancer.

Wheezing was observed in 8 (17.4%) patients, represented in cancers of other localizations 6 (27.3), while with lung cancer in 2 (8.3%) patients.

Occasionally fever was registered in 10 (21.7%)) patients, also more common among patients with cancer of other localizations 6 (27.3%).

Anamnestic positive family history of malignant disease in the family was present at 21 (45.7%) patients, with equal representation (45%) with lung cancer and cancers other sites.

Smoking, emphasized etiologic factor in the development of malignant disease in our study showed no statistical significance of processed data, but notes that current smokers were 22 (47.8%) patients, with equal representation from 45% in lung cancer and other cancers localization in the lungs (Table 2).

For all the associated symptoms of hoarseness was not found statistically significant differences in correlation with the localization of the etiologic process in the lungs and mediastinum, the CT scan and MRI.

Laryngoscopes, in all the examined patients was notable for the limited mobility of the larynx with vocal paramedial position as a sign of recurrent laryngeal nerve. At 41 (89.1%) patients the paralysis is noticed, and at 5 (10.8%) paresis larynx.

More frequent are dextral paralysis of the larynx and in 42 (91.3%) patients, right sided was 4 (8.69%), both sided were not represented.In 22 (91.7%) patients with lung cancer and 19 (86.4%) patients with carcinoma of other sites were represented by unilateral paralysis of the lower laryngeal nerve (Table 3).

Etiological factor leading to recurrent nerve damage diagnosis was confirmed on CT, and then on device for MR-in, which outlines the place of lung cancer as a causal factor of paralysis. Causes of damage to the lower laryngeal nerve in the ENT group examined patients with confirmed diagnosis on CT showed a lung cancer in 14 (58.3%) male and 10 (41.3%) female patients. Tumors of other sites (lung cancer other localization, thyroid cancer, esophageal cancer) were diagnosed in 13 (59.1%) male and 9 (40.9%) female patients.

CT findings indicated the changes that were located in the left lung in 35 (89.74%) patients, right 6 (15.38%) and bilateral in 5 (12.82%) patients. The changes were most often represented in the middle lung fields 24 (61.53%), the hilus 11 (28.2%), upper lung fields 3 (7.69%), the aortopulmonary window, 4 (10.25%) , in the mediastinum 3 (7.69%) and pulmonary peaks 1 (2.56%). Analysis of statistical data showed that there was no statistically significant differences for the parameters and the localization process.

In order to detail the process of differentiation and diagnosis of metastasis in 39 (84.78%) patients had MRI.

Magnetic resonance imaging was done to reduce exposure to radiation and possible chemotherapy, which allowed us to view the mediastinum, the structure of the abdomen and pelvis, thyroid and bone structures of the spinal column, with the aim of establishing a primary focus. This allowed us an insight into soft-tissue structures of the neck and mediastinum, especially in the infiltration of large vessels (aorta, art. Subclavian artery), myocardium and esophagus.

All patients were referred for bronchoscopy which will verify the histological structure of tumor which will be determined by teamwork of oncologists and pulmonologist the course and way of further treatment.

Discussion

Knowing the central, double reciprocal, unilateral and contralateral innervation of the larynx, and ipsilateral peripheral innervation, we conclude that only peripherally vagal influence on the motility of the larynx. Peripheral neurogenic lesions n. laryngeus inferior manifested mostly ipsilateral unilateral vocal cord paralysis. Often this first and dominant character of functional disorders of the larynx leads the patient to the doctor, with the detailed anamnesis we can get more relevant data for further study directed towards the research for etiologic factors and thus enable us to timely diagnosis. [1,4,5] The literature describes one fourth of unilateral laryngeal pa-

ralysis induced by malignant diseases, of which half was caused by lung cancer. Expansive growth of the lung tumor, the rapid creation of regional metastasis to lymph nodes in the region of aortic-pulmonary window, leading to infiltration and compression tree recurrent nerve, result in complete unilateral paralysis of muscles of the larynx. [8,10,11] Because lung cancer is now major cause of death due to cancer, it is necessary to consider its early symptoms - hoarseness and a leading character – vocal paresis for early diagnosis. Considering the issue of the lesion n. laryngeus inferior due to a lung cancer, it is necessary to apply methods for the diagnosis of laryngeal paralysis as a result of nerve damage, as well as all invasive and noninvasive methods for verification of lung cancer as a cause of functional disorders of the larynx. Well-managed comprehensive medical history, standard clinical ENT examination with obligatory indirect laryngoscopes, driven by capital paretic symptom or paralyzed larynx - preliminary results, we can verify the time relative to the oncological problem located in the lungs. Because of that the first caution of the ENT about hoarse patient – is the thought of oncologic aspect of the problem. Since it is usually advanced and inoperable forms of disease, it is reasonable to attempt to identify the natural course of the disease at an early stage for timely and optimal treatment.

Research paresis of recurrent nerve (Walter T. Lee, Milstein C, Hicks D et al.; 2007) indicates hoarseness as the leading symptom, without enlarged lymph nodes, cough and signs of inflammatory processes. Indirect laryngoscopy, bronchoscopy was found vocal cord motility as well as possible morphological changes. The study results show concordance with our results, they have found in all patients typical finding the left recurrent nerve paralysis (vocal in paramedial position), with normal nerve function of the larynx on the right. Based on these findings, inflammatory foci, as well as other morphological changes in the larynx can be excluded, and biopsy is not needed. The combination of left mediastinal mass that reaches to aortic-pulmonary opening and lung tumor localized at the top of the left lung CT findings are often found in patients with unilateral paralysis of the larynx. [12].

Application of high-performance computer technology in the processing of images in the field of radiological technique, computerized tomography (CT) and magnetic resonance imaging (MRI), allow more precise diagnosis of bronchial carcinoma, its localization and relationship with vascular structures of the mediastinum, aortic-pulmonary window, heart, paratracheal space and spinal column, which is relevant for determining the stage of tumor metastatic potential verification process and assess its operability [5,6,9,13]. In our work, guided by previous findings, we followed the main goal - as soon as possible diagnosis of etiological factor of expressed hoarseness due to paralysis of n. laryngeusa inferior, the correlation of clinical findings with the findings of a sophisticated examination of CT or MRI.

Further diagnostics (including fiber and video bronchoscopy) in the direction of determining the histological structure of the tumor as well as access to further treatment (surgical, chemotherapy or radiotherapy), will provide oncologic aspect of the assessment of the problem [16], with a view to the timely commencement of treatment that provides better opportunities for surviving. Epidemiological parameters and symptoms that are displayed in the work, although not shown statistically significant percentage are represented in a large extent (positive family anamnesis, weight loss, fatigue, smoking cigarettes, cough), corresponding to the literature [17] of their direct influence on the development lung cancer diagnosis. Relatively late diagnosis of lung cancer, when diagnosed imaging methods metastatic processes at patient, can be explained by observing a unilateral patients by physicians of different specialties . Then by the ENT observes and examines hoarseness as the leading symptom, and pulmonologists perceive the presence of hemoptysis, dyspnea, cough like symptoms lead to direct further diagnostic imaging methods. To take appropriate diagnostic and therapeutic measures for the treatment of patients with unilateral vocal fold paralysis is required teamwork and cooperation ENT, pulmonologists, radiologists and other specialists.

Conclusion

Hoarseness is the dominant symptom, and laryngeal paralysis dominant sign of grown malignant processs of lung lesions followed by a n.laryngeus inferior. Together with the present pulmonary and

when there isn't one, this symptom associated with characteristic signs is the alarm for otolaryngologist to think of the cause in the chest and in this direction focus further diagnosis. Thorough history and indirect laryngoscopy in the examination indicate that inspiratory stridor with dysplastic dysphonia indicate lung cancer. Diagnosis of the etiologic factors of hoarseness in otolaryngological clinical practice in correlation with modern imaging methods (CT and MRI) will allow the planning of therapeutic procedures (surgery, chemotherapy, radiotherapy). Based on imaging neck multidetector's computed tomography (MDCT) presenting an integral part of preoperative preparation for patients with head and neck cancers. Findings with imaging and complemented with other methods allow for maxillofacial surgeons and otorinolaringologists planning type of neck dissection and other forms of surgical treatment [2].

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Respiratory pathogens and clinical features of acute bronchiolitis in infants

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Abstracts

Objective: We aimed to determine the etiology and clinical characteristics of acute bronchiolitis.

Subjects and methods: Between July 2008 - 2009, children< 2 years of age, admitted to the pediatric emergency unit or pediatric wards of Bulent Ecevit University Hospital, with the diagnosis of acute bronchiolitis were included. Nasopharyngeal aspirates of patients were tested for respiratory viruses by multiplex polymerase chain reaction (PCR) method and the differences between demographic, clinical and laboratory features of patients according to the isolated pathogens were assessed.

Results: During the study 112 patients were followed. Respiratory syncytial virus (RSV) was isolated in 14 (13%) patients, rhinoviruses (RV) in nine (8%), adenovirus in seven (6%), parainfluenza virus-3 (PIV-3) in four (4%) and *Mycoplasma pneumoniae* in one (1%) patient. Polymicrobial etiology was detected in four (4%) patients while in 73 patients (65%) all tested viruses were negative. There was no statistically significant difference between the RSV positive and non-RSV positive groups in symptoms on admission, bronchiolitis scores, duration of hospitalization and duration of symptoms. Relaps was seen in 33 patients and it was statistically more common in non-RSV patients and in the patients who tested negative for all viruses compared with the RSV positive group. (p=0.03)

Conclusion: The use of multiplex PCR for viral detection facilitated the identification of multiple viruses in a single sample. The pathogens that cause acute bronchiolitis do not seem possible to estimate the clinical and laboratory findings.

Key words : Bronchiolitis, infant, respiratory syncytial virus, rhinovirus.

Introduction

Bronchiolitis is an acute, inflammatory respiratory illness of children in the first two years of life and a major cause of hospitalization in that age group. Although bronchiolitis is a common disease that is readily diagnosed clinically, the standard of practice for diagnosis and management of bronchiolitis is still a source of debate (1). RSV is detected in 40-90% of cases of bronchiolitis, and parainfluenza virus (PIV), rhinovirus, adenovirus and influenza are other commonly detected viruses (2-4).

The majority of studies which designed to detect pathogens responsible for bronchiolitis are usually about RSV and used viral culture, rapid antigen tests, immunofluorescence (IFA) method (2-7). However, over the past decade, the development of more sensitive new molecular techniques, such as multiplex polymerase chain reaction (PCR) assays, has increased the number of viruses (e.g., rhinovirus, human metapneumovirus, human bocavirus) detected in children with acute respiratory tract infections (8-10).

Possible differences in the demographic characteristics of infants and in the clinical severity of bronchiolitis in RSV infections and bronchiolitis caused by viruses other than RSV remain controversial. In this study, therefore, we used polymerase chain reaction (PCR)-based methodologies, which offer increased sensitivity for most respiratory viruses, to evaluate the frequency of detection of these agents in acute bronchiolitis and their relationship to the clinical characteristics of the disease.

Patients and methods

This study was conducted prospectively at Bulent Ecevit University Medical School Hospital,

Zonguldak, Turkey; during annual periods from July 2008 through July 2009. All infants (<2 years of age) with acute bronchiolitis, admitted to the pediatric emergency unit or pediatric wards were included. The study was approved by the hospital ethical board and informed consent was obtained from the parents of infants.

Inclusion criteria were final physician diagnosis of bronchiolitis, patient age less than 2 years, and parent informed consent. The diagnosis of bronchiolitis was considered for children younger than 2 years with acute tachypnea, retractions, and abnormal breath sounds who did not meet criteria for a diagnosis of pneumonia or another primary diagnosis (1). Exclusion criteria were underlying chronic diseases (e.g., cystic fibrosis, chronic pulmonary disease, congenital heart disease, and immunodeficiency) and recurrent (more than one) wheezing episodes.

Detailed demographic, clinical and laboratory data were obtained from parents with a structured questionnaire and from patients' medical files. Studied variables included age, gender, month of admission, history of prematurity, breastfeeding history, family smoking habits, number of siblings, family history for asthma and atopy, length of hospital stay, symptoms and signs, need for oxygen therapy evaluated by transcutaneous oxygen saturation, temperature, total white blood count (WBC), C-reactive protein (CRP) serum levels and chest radiological findings. In addition, a clinical severity score ranging from 0 to 12 was assigned to each infant on admission to the hospital according to respiratory rate (<30/min=0, 30-45/min=1, 45–60/min=2, >60/min=3), wheezing (none=0, expiratory with stethoscope=1, expiratory with ear=2, expiratory and inspiratory with ear=3), presence of retractions (none=0, intercostal=1, tracheosternal=2, nasal flare=3), and general appearance (normal=0, anxious=1, anxious and reduced feeding=2, intravenous fluid requirement and altered mental status=3) (11). Telephone interviews were conducted 3, 6 and 12 month after discharged for the development of a new episode. Relapse event was defined as any urgent visit to an emergency department or clinic for wheezing during the 12-months follow-up period.

Virologic study

Nasopharyngeal aspirates were collected using sterile nasopharyngeal swap applicator (microRheologics®), refrigerated at -4ºC within 1 hour and frozen at -80ºC until analysis, which was done within 1 weeks. Multiplex PCR (Seeplex® RV12 ACE Detection) were carried out on every sample for viral pathogens (RSV A/B, hMPV, Parainfluenza 1-2-3, Rhinovirus A/B, Adenovirus, Coronavirus and Influenza A/B viruses) and bacterial pathogens (Mycoplasma pneumoniae, Chlamydia pneumoniae, Bordotella pertussis, Streptococcus pneumoniae, Haemophilus influenzae, Legionella pneumophila) were studied with multiplex PCR (Seeplex® PneumoBacter ACE Detection).

Statistical analysis

Data analysis was carried out with SPSS (version 13.1) for Windows. Data are presented as proportions (with 95% confidence intervals [CI]), means (with standard deviation [SD]), or medians (with interquartile range [IQR]). Clinical characteristics and laboratory variables were compared using Student's t test, Mann-Whitney U test, chi square test, and Fisher's exact test. A one-way analysis of variance (ANOVA) and Student t test were used for the comparison of continuous variables. All p-values are two-tailed, with $p<0.05$ considered statistically significant.

Results

During the study 112 patients were followed. The isolated pathogens were; RSV in 14 (13%) patients, RV in nine (8%), adenovirus in seven (6%), PIV-3 in four (4%) and *M. pneumoniae* in one (1%) patient. Polymicrobial etiology were detected in four (4%) patients; in one patient RSV and RV, in two patients RV and PIV-3 and in one patient coronavirus and adenovirus were isolated concomitantly. In 73 patients (65%) all tested pathogens were negative (Table 1). Human metapneumovirus, PIV 1-2 and Influenza A/B were not isolated from any of the samples.

The number of RSV infections increased in March, although RV was seen all over the year (Figure 1). Participating children had a median age of 7 months (with a range of 1.5 to 24 months) and 63% were male. The demographic characteri-

stics and medical history of the patients are shown in Table 2. Only 27 (24.4%) of the patients were premature. The duration of hospitalization for patients breastfed less than six months was 6.1±5.6 days, while the patients who were breastfed over six months stayed in hospital for 3.4±3.1 days (p=0.049). No statistically significant difference was observed between breastfeeding duration and the severity of the disease (p=0.19).

Table 1. The identified pathogens from children age<2 years presenting with acute bronchiolitis

	N	%
No pathogen identified	73	65,2
Pathogen identified	39	34,8
*RSV A/B**	14	12,5
Rhinovirus	9	8,1
Adenovirüs	7	6,3
PIV-3	4	3,6
RV + PIV-3	2	1,8
RV + RSV	1	0,9
Adenovirus + Coronavirus	1	0,9
Mycoplasma pneumonia	1	0,9

**Two samples are RSV-B.*

There was no statistically significant difference between the groups according to symptoms on admission, bronchiolitis scores, durations of hospitalization and duration of symptoms. The most common symptom was coughing. Fever was observed in 37.5% of the patients. Detection of CRP positivity was significantly more common in the non-RSV group (p=0.03).

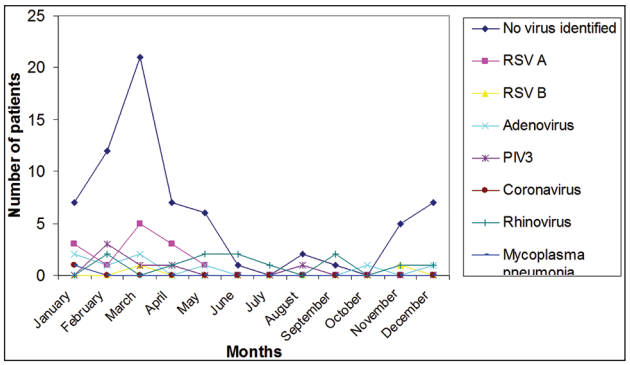

Figure 1. Monthly distribution according to type of virus

Seventeen patients could not be followed so relapse was evaluated for 95 patients. In 33 (35%) of these patients at least one or more bronchiolitis attack was determined. Relaps was seen more common in non-RSV patients and in patients who tested negative for all tested viruses compared to those who tested positive for RSV (p=0.03). There was no statistically significant relation between the presence of an allergic disease in a family member and the rate of recurrence (p=0.07).

Discussion

The findings of this prospective study on infants hospitalized with bronchiolitis suggest that bronchiolitis is a well-characterized clinical entity that can be associated with various viral pathogens. It was found that the three most common pathogens were RSV (13%), RV (8%) and adenovirus (6%). It was striking that the causative

Table 2. Demographic factor and medical history among children with bronchiolitis

	Total n=112	RSV n=14	Non-RSV* n=25	No Identified Virus n=73	P
Age (month), (mean±SD)	8,4±5,8	7.5±6,5	10±5,6	8,1±5,7	0.076
<6 months, n (%)	48 (42.9)	9 (64.3)	7 (28.0)	32(43.8)	
6-12 months, n (%)	43 (38.4)	2 (14.3)	13 (52.0)	28 (38.4)	
13-18 months, n (%)	11 (9.8)	2 (14.3)	2 (8.0)	7 (9.6)	
19-24 months, n (%)	10 (8.9)	1 (7.1)	3 (12.0)	6 (8.2)	
Male, n (%)	71 (63.4)	11 (78.6)	16 (64.0)	44 (60.3)	0.42
Premature, n (%)	27 (24.1)	3 (2.7)	5 (4.4)	19 (17.0)	0.80
Breastfed (< 6 months), n (%)	69 (61.6)	11 (78.6)	10 (40.0)	48 (65.8)	***0.028***
Has siblings in home, (mean±SD)	2.1±1.2	1.7±0.5	2.5±1.1	2.0±1.2	0.07
Any parental history of asthma or atopy, n (%)	34 (30.4)	4 (28.6)	9 (36.0)	21 (28.8)	0.374
Exposure of smoking, n (%)	58 (48.2)	7 (50.0)	10 (40.0)	41 (56.2)	0.785

**Mix infections labeled to non-RSV.*

Table 3. Clinical manifestations of acute bronchiolitis: RSV and non-RSV children

	Total n=112	RSV n=14	Non-RSV n=25	No Identified Virus n=73	p
Severity of bronchiolitis (Bronchiolitis score ± SD)	5.6±1.6	5,8±1,7	5,8±1,5	5,5±1,6	0.64
Oxygen saturation on room air at enrollment, mean ± SD	94.0±3.3	95.2±2.5	93.6±3.5	93.9±3.4	0.41
Duration (in days) of symptoms	12.5±7.9	14.2±11.7	12.6±7.4	12.1±7.2	0.75
Presence of cough (%)	97.3	100	96.0	97.3	0.75
Presence of wheezing (%)	93.8	92.9	88.0	95.9	0.408
Presence of fever (%)	37.5	28.6	44.0	37.0	0.627
Presence of rhinorrhea (%)	58.9	57.1	48.0	63.0	0.41
Presence of vomiting (%)	20.5	14.3	24.0	20.5	0.77
Presence of retraction (%)	55.4	57.1	44.0	58.9	0.42
Presence of cyanosis (%)	5.4	14.3	0.0	5.5	0.16
Hospitalization, n (%)	88 (78.6)	9 (64.3)	21 (84.0)	58 (79.5)	0.33
Mean (SD) length of stay, days	5.1±4.9	5,2±8,4	6,1±4,5	4,7±4,2	0.17
Re-wheezing or readmission within one year*, n (%)	33 (34,7)	1 (8.3)	11 (52.4)	21 (33.9)	0.03
WBC count, mean±SD	12.6±5.8	12.5±5.2	13.0±5.6	12,5±6,0	0.85
Lymphocytosis, mean±SD	5.8±4.0	5.8±3.2	4.2±2.0	6.4±٤,٥	0.121
CRP positive (>3.0 mg/ dl) (%)	34.9	18.2	57.1	29.4	0,03

** Relapse event were evaluated for 95 patients.*

pathogen remained undetected in a large percentage of patients. PCR, a sensitive and comprehensive technique, was employed in this study and both sampling and storing were done meticulously, but technical issues, presence of other viral factors and the possibility of allergy-related wheezing might be responsible for the high rate of cases that tested negative for all viruses.

RSV is the leading cause of bronchiolitis, being responsible for 50-90% of all cases around the world (3,12). However, studies conducted in Turkey suggest that only 11-43% of all cases of bronchiolitis are caused by RSV (2,4,5). This study confirms that RSV is the most common pathogen found in patients with bronchiolitis. Bronchiolitis caused by RSV follows a seasonal pattern and is seen most in early winter and early spring, while being almost nonexistent during fall and summer. It was seen that rhinovirus, which is the second leading cause of bronchiolitis, could lead to disease in all seasons. Earlier studies conducted using the PCR technique showed that rhinovirus is not only a cause of higher respiratory diseases but it is also responsible for as high as 29% of lower respiratory diseases (13,14). Human metapneumovirus, which was first identified in 2001 in patients with respiratory diseases in the Netherlands, is reported as a cause of respiratory tract infections in many countries (15,16,17). This virus is seen in 4-9% of the 0-2 aged patients with lower respiratory tract infections (18). However in our study, hMPV was not identified in any of the patients. This could be explained by technical issues, the relatively small size of the sample and the fact that the sample consisted only of patients with bronchiolitis. Other reasons could be the fact that hMPV might not be one of the major cause of respiratory tract diseases in Turkey. It could also have to do with the fact that parainfluenza type 1-2 and influenza becomes epidemic once in every two years and that they are a rare cause of bronchiolitis.

Another important finding of this study was that no significant difference was found between RSV positive and non-RSV viruses in demographic characteristics except age and seasonal prevalence patterns and in the clinical severity of the disease. Similarly, no significant differences in demographic characteristics were detected between patients who tested RSV positive and those who tested negative for all viruses.

Earlier studies suggest that rhinovirus leads to increased clinical severity and is associated with atopy (14). It was also suggested that rhinovirus and mixed infections result in prolonged duration

of hospitalization and that RSV has a milder clinical severity (8, 14). The findings of this study do not confirm these claims but rather indicate that the presence of virus has no effect on the clinical severity of bronchiolitis.

Recurring wheezing attacks might be observed in 40-50% of the patients hospitalized due to acute bronchiolitis. In some infants the recurrence of attacks could continue until they are 2-3 years old (19). In Valkonen et al. study that involved patients with bronchiolitis followed for three years, the rate of recurrence was 9%, 15% and 18% during the first, second and third year respectively. In this study the authors grouped patients as, RSV positive and RSV negative, and they found that the rate of recurrence for RSV negative patients was higher at the end of the third year (27.2%) compared to RSV positive patients (8%) (20). In our study; the disease recurred in 34.7% of the patients that were followed for one year. The patients who tested negative for all viruses and those who tested positive for a virus other than RSV had a higher rate of recurrence. The rate of recurrence was high especially in patients with rhinovirus. This study did not specifically examine the patients for allergy and atopy, but their family histories were collected. It was found that a family history of atopy did not constitute a risk factor for the recurrence of the disease. Future studies that involve examining infants in detail for atopy, conducting skin tests and assessing the level of eosinophil will further illuminate the question of recurrence.

In conclusion; our study confirms the previous observations that RSV is the most frequently detected virus in patients with bronchiolitis, and it also highlights the potential significance of other viral pathogens such as rhinovirus and adenovirus for bronchiolitis. Although it is not possible to estimate the etiologic agent of acute bronchiolitis from clinical and laboratory findings, infants older than one years old and who are admitted during summer or early fall are most likely to be infected with non-RSV viruses. The use of multiplex PCR for viral detection facilitated the identification of multiple viruses in a single sample.

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Characterization of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae isolated from clinical specimen in an Iranian Pediatric Hospital

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Abstract

Background and objectives: Extended spectrum B-lactamase (ESBL)-producing organisms pose unique challenges to clinical microbiologists and clinicians. Although ESBLs are a global problem, specific enzymes and genotypes can be different in geographic regions, hospitals and individual hospital units. Knowledge of local resistance patterns is an important issue for clinicians. The aim of this study was to describe phenotypic and genotypic characteristics and antibiotic sensitivity of ESBLs in a pediatric hospital in Iran.

Methods: One-hundred and eighty six extended-spectrum beta-lactamase (ESBL) producer *E. coli* and *Klebsiella pneumoniae* strains isolated from hospitalized children in an Iranian pediatric hospital. All isolates were analyzed for their susceptibility testing, presence of AmpC genes and their genetic patterns.

Results: A total of 186 isolates of ESBL producer *Ecoli* (82.8%) *and K. pneumoniae* (17.2%) were obtained from urine (62.4%), blood (16.1%) and stool (21.5%) samples. The rate of *bla*TEM, *bla*CTX-M, *bla*SHV and *blaPER* genes in the isolates were 154(82.8%), 147(79%), 55(29.6%) and 22(11.8%) respectively. Imipenem and meropenem were the most effective antibiotics against ESBLS isolates. The rates of resistance to other antibiotics were as follows: co-trimoxazole (83.9%), ciprofloxacin (47.3%), amikacin (36.6%) and gentamicin (33.9%).

Conclusion: In our hospital, the most frequent gene was *bla*TEM. According to the antibiotic susceptibility testing results, carbapenems are the best choice antibiotics for treatment of infections caused by ESBL producing organisms.

Key words: E.coli, K. pneumonia, ESBLs.

Introduction

Resistance to β-lactam antibiotics has increased significantly in the last two decades and has been documented in both community and hospital settings [1, 2, 3 4]. Resistance to expanded spectrum beta-lactams has been found among the strains of *K. pneumoniae* and *E. coli.* Isolates that produce extended spectrum beta-lactamase (ESBLs) are resistant to penicillins, extended spectrum cephalosporins, monobactams and aztreonam [5, 6].

In Enterobacteriacae family, the main beta-lactamase enzymes are TEM and SHV. Within a few years of the commercial release of β-lactam antibiotics, gram -negative bacilli that harbored mutated versions of the potent TEM and SHV enzymes were detected. These and other newly detected β-lactamases (for example CTX-M and PER types) hydrolyze β-lactam antibiotics containing the oxymino side-chain. AmpC β-lactamases are able to hydrolyse monobactams, cephamycins and cephalosporins and are not inhibited by ESBL inhibitors such a clavulanic acid [7]. Incidence of these resistant factors and multiple β-lactamase producers which are able to produce different types of ESBLs and AmpC can cause serious problems in future regarding the treatment of infections caused by this organisms [8]. Many ESBL producers also carry other genes (such as CTX-M, TEM, etc.) that confer resistance to other antimicrobial agents such as aminoglycosides and fluoroquinolones [9, 10].

With reports on high prevalence of ESBLs production in *E. coli* and *K. pneumoniae* and according to paucity of information about these isolates in Iran, especially in children, the present study was carried out to determine the prevalence of the

genes encoding SHV, TEM, CTX-M, PER and AmpC in ESBL producing *K. pneumonia* and *E. coli* and also to determine the pattern of antibiotic resistance among these isolates in order to reduce the usage of inappropriate antibiotic therapy.

Materials and methods

Bacterial strains

In this analytical cross-sectional study, we assessed 186 isolates of ESBL producer *E.coli* (n=154) and *K. pneumoniae* (n=32) which were isolated from the clinical specimens including blood (n= 30), urine (n= 116) and stool (n=40) of patients admitted to Mofid Children Hospital, Tehran, Iran, 2011.

Detection of extended spectrum beta lactamases (ESBL)

Production of ESBLs in our study among *E. coli* and *K. pneumoniae* isolates was determined by the combination disk technique using antibiotic disks that was recommended by the clinical laboratory standard institute (CLSI 2010)

Antimicrobial susceptibility

Susceptibility testing to other antibiotics was performed by disk diffusion methods as recommended by CLSI 2010. The following antibiotic disks (MAST diagnostic Group UK) were used: Gentamycin (10 µg), Amikacin (30 µg), Co-trimoxazol (1.25/23.75 µg), Imipenem (10 µg), Meropenem (10 µg), Ciprofloxacin (5 µg).

Extraction and amplification DNA

Isolates producing ESBLs were subjected to polymerase chain reaction (PCR) targeting *bla*SHV, *bla*TEM, *bla*CTX-M, *bla*PER and *bla*AmpC genes. Genomic DNA was extracted by phenol/chloroform method [15]. PCR amplification was performed using the primers listed in Table 1 and 2; the primers were obtained from Genet Bio Inc., Iran.

Table 1. Primer sequences of the ESBL genes amplified by PCR.

SHV-A	AAGATCCACTATCGCCAGCAG
SHV-B	ATTCAGTTCCGTTTCCCAGCGG
TEM-A	GAGTATTCAACATTTCCGTGTC
TEM-B	TAATCAGTGAGGCACCTATCTC
PER-A	ATGAATGTCATTATAAAAGC
PER-B	AATTTGGGCTTAGGGCAGAA
CTX-MA	CGCTTTGCGATGTGCAG
CTX-MB	ACCGCGATATCGTTGGT

Table 2. Primer sequences of the AmpC genes amplified by PCR

AmpC	
FoxMF	AACATGGGGTATCAGGGAGATG
FoxMR	CAAAGCGCGTAACCGGATTGG
ACCMF	AACAGCCTCAGCGTAACCG-GATTGG
ACCMR	TTCGCCGCAATCATCCCTAGC
DHAMF	AACTTTCACAGGTGTGCTGGGT
DHAMR	CCGTCGCATACTGGCTTTGC

Data analysis

All data were analyzed with SPSS12 software for Windows (SPSS Inc., Chicago, IL). *P* values less than 0.05 assumed significant.

Results

A total of 186 isolates of *ESBL producer* organisms were assessed of which 154 isolates were *Ecoli* (82.8%) and 32 isolates were *K. pneumoniae* (17.2%). We had 116 specimens of urine (62.4%), 30 specimens of blood (16.1%) and 40 specimens of stool (21.5%). These were samples which were submitted to the microbiology laboratory in Mofid Children Hospital.

Antibacterial resistance pattern

Imipenem and meropenem were the most effective antibiotics against ESBL producing isolates and respectively only10 isolates (5.4%) and16 isolates (8.8%) were resistant to imipenem and meropenem.

The rates of resistance to other antibiotics are demonstrated in Figure 1.

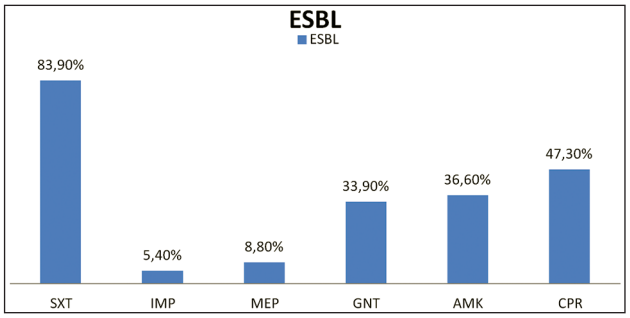

Figure 1. The pattern of antibiotic resistance in ESBLs isolates. SXT: Cotrimoxazol, IMP: Imipenem, MEP: Meropenem, GNT: Gentamycin, AMK: Amikacin, CPR: Ciprofloxacin

Detection of ESBLs genes by PCR

The rate of *bla*TEM, *bla*CTX-M, *bla*SHV, and *blaPER* in the isolates were 154(82.8%), 147(79%), 55(29.6%), and 22(11.8%) respectively. In our study, 52 isolates contained AmpC genes totally and 45 (29.2%) of Ecoli isolates and 7 (21.9%) of *Klebsiella pneumoniae* isolates contained AmpC type β-lactamases.

The prevalence of simultaneous presence of *bla*TEM, *bla*SHV, *blaPER*, *bla*CTX-M and AmpC are shown in Table 3.

The prevalence of SHV gene among *Ecoli* isolates was 24% and in Klebsiella isolates was 56.3%, ($p<0.05$). The prevalence of PER gene among E.coli isolates was 14.3% and in Klebsiella isolates was 0%, ($p<0.05$). The prevalence of genes in each group of germs are shown in Table 4.

In urine samples 37.9% and 7.5% of stool specimens were positive for SHV ($p<0.05$). In urine specimens 17.2% and 6.7% of blood specimens were positive for PER, ($p<0.05$). The prevalence of different genes among different specimens is presented in table 5 and the relationship between the genes and antibiotics resistance are mentioned in table 6. There was a significant association between presence of SHV, PER and resistance to amikacin. Also we found a significant relationship between absence of SHV, CTXM and resistance to Co-trimoxazol and ciprofloxacin, respectively.

Discussion

During the past decade, ESBLs producing gram-negative bacilli especially *E. coli and K. pneumoniae* have emerged as serious pathogens both in hospital and community acquired infections worldwide [11]. On the matter of antibiotic resistance we found that the most effective antibiotics on ESBL producer organisms were carbapenems, as 94.6% and 91.2% isolates were susceptible to imipenem and meropenem respectively. Ullah et al. from Pakistan reported that 93.48 and 86.96% isolates were susceptible to meropenem and imipenem respectively [12]. This was consistent with findings of Al-Zahrani and Akhtar from Saudi Arabia [13].

In our study, resistance to gentamycin and amikacin was 33.9% and 36.6% respectively. Other researchers have reported overall full resistance rates, (intermediate plus resistant isolates) of ESBL organisms to aminoglycoside compounds as follows: amikacin, 6.7%; gentamicin and tobramycin, 27.4%;

Table 3. The prevalence of genes in isolates

Positive all five genes	0.5%	CTX-M, PER	1.1%
TEM, SHV, PER, CTX-M	3.2%	CTX-M, TEM	31.7%
TEM, AmpC, PER, CTX-M	2.2%	CTX-M, SHV	2.2%
CTX-M, SHV, TEM, AmpC	4.3%	CTX-M, AmpC	1.6%
TEM, CTX-M, PER	2.7%	PER, TEM	1.1%
CTX-M, PER, SHV	0.5%	PER, SHV	0.5%
CTX-M, SHV, TEM	12.9%	TEM, SHV	2.7%
CTX-M, TEM, AmpC	10.8%	TEM, AmpC	3.2%
CTX-M, SHV, AmpC	0.5%	SHV, AmpC	1.1%
TEM, SHV, AmpC	0.5%	negative for all genes	1.1%

Table 4. The prevalence of genes in each microorganism

	TEM	PER	SHV	CTX-M	AmpC
Ecoli	(81.8%)126	22(14.3%)	37(24%)	120(77.9%)	45(29.2%)
Klebsiella	28(87.5%)	0	18(56.3%)	27(84.4%)	7(21.9%)

Table 5. The prevalence of genes among different specimens

	TEM	PER	CTX-M	SHV	AmpC
UC	85.3%	17.2%	82.8%	37.9%	26.7%
BC	80%	6.7%	70%	26.7%	26.7%
SC	77.5%	0.0%	75%	7.5%	32.5%

UC: Urine culture, BC: Blood culture, SC: Stool culture

Table 6. The relationship between genes and antibiotic resistance

		SXT	IMP	MRP	GNT	AMK	CPR
TEM	Positive	85.7%	5.8%	9.9%	31.2%	37.7%	46.1%
	Negative	75%	3.1%	3.2%	46.9%	31.3%	53.1%
	P value	0.134	0.535	0.230	0.088	0.493	0.469
SHV	Positive	72.7%	9.1%	9.1%	43.6%	50.9%	54.5%
	Negative	88.5%	3.8%	8.7%	29.8%	30.5%	44.3%
	P value	0.007	0.146	0.925	0.068	0.008	0.200
PER	Positive	81.8%	4.5%	4.5%	36.4%	72.7%	50%
	Negative	84.1%	5.5%	9.4%	33.5%	31.7%	47%
	P value	0.780	0.854	0.453	0.792	0.000	0.788
CTX-M	Positive	84.4%	4.8%	9.7%	34.7%	39.5%	42.9%
	Negative	82.1%	7.7%	5.3%	30.8%	25.6%	64.1%
	P value	0.728	0.471	0.388	0.645	0.111	0.018
AmpC	Positive	86.5%	7.7%	8.2%	34.6%	44.2%	57.7%
	Negative	82.8%	4.5%	9%	33.6%	33.6%	43.3%
	P value	0.538	0.383	0.856	0.894	0.176	0.077

kanamycin, 26.3%; and streptomycin, 50.5% [14].

Since ESBL encoding genes are generally found on plasmids, many of the organisms that harbor ESBLs also are resistant to different classes of antibiotics such as aminoglycosides, flouroquinolons, tetracyclines, chloramphenicol and sulfonamides. The observed resistance to ciprofloxacin in our study was 47.3%, although this rate was reported up to 55.8% in the Lutenbach study [15]. Some factors such as fluoroquinolone use, aminoglycoside use and long-term care facility residence could influence the rate of this kind of resistance.

We recorded 83.9% resistance to co-trimoxazole. In the study by Behroozi et al approximately 76% of klebsiella and Ecoli ESBL producers were resistant to cotrimoxazol [11]. Another study from Oman reported 86% resistance to cotrimoxazole in ESBL producing organisms [16]. These results, which are similar to Jett et al's study [17], highlight the fact that many of ESBL organisms are resistant to non-betalactam antibiotics such as co-trimoxazole, ciprofloxacin or gentamicin.

ESBL genes

The prevalence of *bla*SHV and *bla*TEM genes in this study was 29.6% and 82.8%, respectively which is different from the results of the multi-national study group, (67.1% and 16.4% respectively) [18].

The number of CTX-M type ESBLs is rapidly expanding. They have been detected in some geographic areas and are now the most frequent ESBL type worldwide [19]. In our study, the prevalence of *bla*CTX-M was 79%. Edelstein et al reported 47.2% of *Enterobacteriaceae* having CTX-M-type -lactamases and these were the most common ESBLs in Russian ICUs [20].

Also in another study from South America where the ESBL rates are among the highest in the world, SHV- and TEM-type ESBLs have been frequently encountered, but CTX-M is endemic and widely dominant. PER-type ESBLs seem to be restricted to the southern 'cone' of South America [21].

In a study from Iran in 2011 the frequency of SHV and TEM among the ESBL producing isolates were 14.4% and 20.6%, respectively [22]. Although in this study the rate of TEM was greater than SHV but it was lower than our results, (82.8% in compare to 20.6%). Other studies show different incidence of *bla* genes in Iran [23-26]. This may be due to many factors such as different kind of organisms that were surveyed, different populations/locales and also differences in methodology.

Although all these studies are based on the PCR method but as Sharma et al reported amplification of whole genomic DNA increases the likelihood of detection, compared to amplification of plasmid DNA or chromosomal DNA alone, [27].

In 2003 prevalence of *bla*PER gene in Turkey was 55.5% while in 2006 this gene was not isolated in studies from Italy [28]. Also we found that

14.3% of E.coli isolates were positive for bla PER but none of our *Klebsiella* isolates contained the mentioned gene.

It is obvious that genetic determinants for AmpC β-lactamases which were commonly found on the chromosomes of genera such as *Enterobacter* and *Citrobacter,* have now been transferred on to plasmids and have spread to other organisms, including *E. coli* and *Klebsiella*; this type of transmission is very important because it increases the prevalence of resistant organisms. In our study, among the isolates that contained AmpC gene, 96.2% were resistant to cefpodoxim, 96.2% were resistant to ceftazidime and 94.1% were resistant to ceftriaxone.

As patients infected with ESBL producers are at increased risk of treatment failure with extended spectrum-beta-lactams, the presence of AmpC is important because it influences the therapeutic options.

AmpC was detected in 29.2% of Klebsiella sp in our study However, *Klebsiella* spp. do not possess a chromosomal AmpC. Also, it was shown that AmpC enzymes were not susceptible to inhibitor combinations especially in Plasmid-mediated AmpC β-lactamases that are present in *Klebsiella* sp. It is a new threat since they confer resistance to 7-α-methoxy-cephalosporins such as cefoxitin or cefotetan, are not affected by commercially available β-lactamase inhibitors, and can, in strains with loss of outer membrane porins, provide resistance to carbapenems [29]. Especially Glasgow study shows almost 14% of Enterobacteriaeceae are potential AmpC/ESBL producers [30], which unfourtunaitly was 28% in our study.

Conclusion

In view of the antibiotic resistance pattern among ESBL producer bacterial pathogens carbapenems could be the best choice to decrease treatment failure in managing infections caused by these organisms. Because of capability of ESBL organisms in harboring different genes with special effects on antibiotic sensitivity it is particularly important to take great care in ascertaining the antibiotic sensitivity of these organisms.

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Is there a relationship between severity of pulmonary disease obstruction and Helicobacter pylori infection?

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Abstract

Background and aims: It has been shown by seroepidemiological studies that there is an association between *Helicobacter pylori* (Hp) infection and some respiratory system disorders. At present, data about the possible relationship between bacterial load and severity of the respiratory obstruction are insufficient. This study aimed to investigate the relationship between bacterial load and severity of obstruction in patients with obstructive pulmonary disease and Hp infection.

Methods: The study group included a total of 380 patients with Bronchial asthma (BA) (n: 188, Group 1) or Chronic obstructive pulmonary disease (COPD) (n: 192, Group 2). Spirometry and C14-urea breath tests were done. The patients were classified in four stages according to international criteria as follows: Group 1 (Global Initiative for Asthma (GINA) criteria; 53/188 in stage 1, 58/188 in stage 2, 51/188 in stage 3, 26/188 in stage 4) and Group 2 (Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria; 35/192 in stage 1, 35/192 in stage 2, 62/192 in stage 3, 60/192 in stage 4).

Results: Regarding distribution of Hp load, there were no significant differences between stages in Group 1 or between stages 1, 2 and 3 in Group 2 ($p>0.05$). However, a significant difference was determined between stage 4 and the other stages in Group 2 ($p<0.05$). When the same stages of the two groups were compared, there was a difference only between stage 4 patients ($p<0.05$).

Conclusions: There was a relation for only high stage (stage 4) COPD patients about bacterial load and severity of obstruction, but there was no any relation for all stage of patients with BA.

Key words: Chronic obstructive pulmonary disease (COPD), Helicobacter pylori (Hp), Pulmonary function tests, C14-urea breath test

Introduction

Today, nearly half of the world's population has been infected by *Helicobacter pylori* (Hp), which is a microaerophilic, gram-negative bacteria (1). Hp infection plays a basic role in the pathogenesis of chronic gastritis and peptic or duodenal ulcer, and it is even considered as a risk factor for the development of gastric carcinoma (2). Hp prevalence was reported to be higher in various extra-gastroduodenal pathologies, such as cardiovascular, dermatological, hepatic, and endocrine-metabolic diseases (3-6). In these systemic disorders, Hp infection was thought to contribute to the pathogenesis of the disease through the abnormal stimulation of some inflammatory mediators and/or autoimmunity (7-9). Some authors have studied the possibility of a connection between Hp infection and several respiratory system disorders, including chronic obstructive pulmonary disease (COPD), bronchial asthma (BA), bronchiectasis, interstitial lung disease, pulmonary tuberculosis, and lung cancer (10-12). Some authors obtained significant evidence for a relationship between Hp infection and COPD but not BA (12-14). Although at present the data in patients with COPD are insufficient, it was reported that Hp infection might also be related to the severity of the obstruction (15).

In this prospective study, we investigated the possible relationship between Hp load and severity of obstructive pattern by using staging systems and a comparative method.

Materials and methods

Subjects

Totally 380 patients (250 female, 130 male, mean age ± SD, 49.0 ± 15.2 years) admitted to Ankara Training and Research Hospital and

Yildirim Beyazit Training and Research Hospital of the Ministry of Health were enrolled in this study. The patients were queried with respect to diagnostic criteria, medical therapy, adherence to treatment, times of hospitalization and emergency service visits in the last year, atopic or dyspeptic complaints (abdominal pain, nausea, vomiting episodes, etc.), presence of chronic symptoms (dyspnea, cough, sputum, chest pain, etc.), smoking history, and socioeconomic status during childhood. A detailed clinical evaluation was conducted including physical examination, chest X-ray, routine hematologic and biochemical tests, spirometric tests, and C14-urea breath test (UBT). Direct examination, gram staining, microbiological culture, and antibiogram studies were performed in patients with chronic sputum production. Upper respiratory tract infections or obstructive attacks within the last three months and diagnosis of a community-acquired pneumonia, malignancy or severe cardiovascular disease were used as the exclusion criteria. This study was approved by the local ethical committee of Ankara Training and Research Hospital. All patients provided their oral/written informed consent.

Patients' characteristics

The patients were categorized in four stages with respect to spirometric test results in two groups with obstructive disease. Group 1 comprised 188 patients with BA (188/380, 49%). The number and mean age ± standard deviation (SD) of the patients according to the stages were 53/188 (28%) and 35.7 ± 10.1 y in stage 1, 58/188 (31%) and 34.9 ± 12.6 y in stage 2, 51/188 (27%) and 39.9 ± 9.3 y in stage 3, and 26/188 (14%) and 43.6 ± 9.2 y in stage 4. Group 2 consisted of 192 patients with COPD (192/380, 51%). Distribution of the patients into stages according to number and mean age ± SD were: 35/192 (18%) and 60.3 ± 8.8 y in stage 1, 35/192 (18%) and 58.3 ± 8.5 y in stage 2, 62/192 (32%) and 59.3 ± 8.2 y in stage 3, and 60/192 (32%) and 62.7 ± 9.6 y in stage 4. The characteristics of the patient population are summarized in Table 1.

C14-urea breath test

C14-UBT is a non-invasive and reliable method with high sensitivity and specificity for detecting Hp infection (16). Each patient was checked using the C14-UBT. The patients were fasted at least 6 hours before the test. The patients were asked to brush their teeth 20 minutes before the test. Antibiotics, proton pump inhibitors and antiacid drugs/H2 receptor antagonists were stopped at least one month, 14 days and 24 hours, respectively, before the test. None of the patients was pregnant or nursing. No patient in either group had undergone gastric resection. Routine protocol was used for the test. Ten minutes after swallowing a capsule of urea labelled with 1 μCi (37kBq) of 14C (Helicap, Noster System AB, Stockholm, Sweden) with a glass of water (25-50 mL), patients were asked to breathe into a breath collector (BreathCard, Kibion, Stockholm, Sweden) to pro-

Table 1. The characteristics of the patient population for the four stages and two groups

Groups/Stages	Subjects		Gender		Age
	No. (n)	Percent (%)	Female (n)	Male (n)	Mean ± SD*
Group 1					
Stage 1	53/188	28	41	12	35.7 ± 10.1
Stage 2	58/188	31	46	12	34.9 ± 12.6
Stage 3	51/188	27	35	16	39.9 ± 9.3
Stage 4	26/188	14	21	5	43.6 ± 9.2
Total	188/380	49	143	45	37.4 ± 11.0
Group 2					
Stage 1	35/192	18	19	16	60.3 ± 8.8
Stage 2	35/192	18	22	13	58.3 ± 8.5
Stage 3	62/192	32	32	30	59.3 ± 8.2
Stage 4	60/192	32	34	26	62.7 ± 9.6
Total	192/380	51	107	85	60.3 ± 8.9

**SD: Standard Deviation*

vide an adequate breath sample. The results were calculated by a Heliprobe analyzer (Kibion AB, Uppsala, Sweden) over 250 seconds. The unit of measurement was counts per minute (cpm).

Spirometry test and the staging of obstruction severity

A spirometry test was carried out in all patients using a SensorMedics Vmax 29 spirometry system (SensorMedics, Yorba Linda, CA) with the patients in sitting position. The analyzer was calibrated prior to each study. The measurements were compared with the predicted values. The best flow-volume loop was used for the analysis of the data. Forced expiratory volume in one second [FEV1 (L,%)], forced vital capacity [FVC (L,%)], FEV1/FVC ratio (%), and peak expiratory flow [PEF (L/n,%)] values were used to determine the airflow disturbance. Severity of airway obstruction was defined in four stages by spirometry criteria according to following guidelines. Group 1 patients were classified as intermittent (Stage 1; FEV1 or PEF ≥ 80% predicted, PEF variability < 20%), mild persistent (Stage 2; FEV1 or PEF ≥ 80% predicted, PEF variability 20-30%), moderate persistent (Stage 3; FEV1 or PEF 60-80%, PEF variability > 30%), and severe persistent (Stage 4; FEV1 or PEF ≤ 60%, PEF variability > 30%) according to the criteria of the Global Initiative for Asthma (GINA) guidelines. Group 2 patients were categorized as mild (Stage 1; FEV1 ≥ 80% predicted, FEV1/FVC < 70%), moderate (Stage 2; FEV1 50-80% predicted, FEV1/FVC < 70%), severe (Stage 3; FEV1 30-50% predicted, FEV1/FVC < 70%), and very severe (Stage 4; FEV1 ≥ 30% predicted or FEV1 < 50% predicted plus chronic respiratory failure, FEV1/FVC < 70%), taking into account the criteria included in the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines.

Statistical analysis

Statistical analyses were performed with the SPSS software program (Version 11.5.0, SPSS Inc, Chicago, IL, USA). Suitability of the normal distribution of data was evaluated using the Shapiro-Wilk test. In order to make comparisons between the stages of the disease, one-way variance analysis and Kruskal-Wallis variance analysis methods were used for variables showing normal and abnormal distribution, respectively. Data were summarized as mean ± SD or median (minimum-maximum) with respect to the analysis method used. A p value <0.05 was considered significant.

Results

Group 1

Mean ages ±SD were 35.7 ± 10.1 y in stage 1, 34.9 ± 12.6 y in stage 2, 39.9 ± 9.3 y in stage 3, and 43.6 ± 9.2 y in stage 4. The distribution of the spirometry test results according to stages was as follows: The median (min-max) values for FVC, FEV1 and PEF measurements were 3.02 (2.2-5.7), 2.6 (1.7-4.4) and 5.6 (2.3-10.2) in stage 1, 2.7 (1.2-4.4), 2.1 (1.0-3.4) and 4.3 (1.9-8.1) in stage 2, 1.8 (0.9-3.4), 1.3 (0.7-2.4) and 2.9 (1.0-5.7) in stage 3, and 0.9 (0.5-2.1), 0.7 (0.4-1.3) and 1.9 (0.4-3.8) in stage 4, respectively. The mean ± SD values for FEV1/FVC were calculated as 85.1 ± 6.5 in stage 1, 78.2 ± 8.8 in stage 2, 72.3 ± 8.2 in stage 3, and 74.2 ± 13.8 in stage 4. The median (min-max) values for C14-UBT measurements were 19 (1-408) in stage 1, 97.5 (2-455) in stage 2, 21 (2-577) in stage 3, and 17 (1-370) in stage 4. There were no statistically significant differences between any of the stages in terms of the C14-UBT results (p>0.05).

Group 2

Mean ages ±SD were 60.3 ± 8.8 y in stage 1, 58.3 ± 8.5 y in stage 2, 59.3 ± 8.2 y in stage 3, and 62.7 ± 9.6 y in stage 4. The spirometry test results revealed that the median (min-max) values for FVC, FEV1 and PEF were 2.6 (1.1-4.8), 2.1 (0.9-4.4) and 5 (3.0-11.9) in stage 1, 2.3 (1.4-4.6), 1.8 (1.1-3.3) and 4.3 (1.2-8.9) in stage 2, 1.7 (0.8-3.4), 1.2 (0.7-2.7) and 2.7 (1.1-6.2) in stage 3, and 1.2 (0.4-2.9), 0.7 (0.4-2.3) and 1.4 (0.6-5.2) in stage 4, respectively. The mean ± SD values for FEV1/FVC were 84.9 ± 7.6 in stage 1, 80.6 ± 8.1 in stage 2, 72 ± 10.5 in stage 3, and 68.1 ± 15.1 in stage 4. The median (min-max) values for the C14-UBT were calculated as 19 (4-430) in stage 1, 76 (2-383) in stage 2, 19.5 (1-484) in stage 3, and 132.5 (1-541) in stage 4. In terms of C14-UBT, a statistically significant difference was found between stage 4 and the other stages (p<0.05); however, there was no statistically significant difference between stages 1, 2 and 3 (p>0.05).

When Group 1 and 2 patients in the same stages were compared in terms of C14-UBT results, we determined that there was a statistically significant difference for stage 4 patients ($p<0.05$), but not between patients in the other stages ($p>0.05$).

All results related to the pulmonary function tests and C14-UBTs in Groups 1 and 2 are presented in Table 2 and Figure 1.

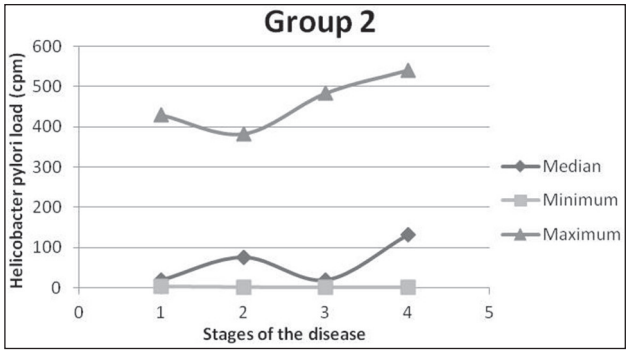

Figure 1. The distribution trend curves according to the stages for helicobacter pylori load (cpm) in group 2 patients.*
*(*cpm; count per minute)*

Discussion

Helicobacter pylori infection is a global health problem and is associated with various extra-gastroduodenal pathologies. Seroprevalence studies for Hp infection in the obstructive lung diseases such as BA and COPD have been published in the last decade (11-15). However, to the best of our knowledge, a possible relationship between Hp infection and the severity of the obstructive pattern has not been investigated in detail previously.

Bronchial asthma is a disorder caused by periodic, reversible constriction of the bronchi. Some authors have revealed a poor connection with Hp infection. Tsang et al. (12) showed that there was no statistically significant difference between the BA group (47.3%) and a healthy control group (38.1%) with respect to Hp seroprevalence, and further, that serum Hp IgG levels were not related to FEV1 % predicted, FVC % predicted or duration of asthma ($p>0.05$). Jun et al. (13) investigated anti-Hp IgG and anti-Hp cytotoxin-associated gene A (CagA) seropositivity in three groups of patients. They did not find a statistically significant differ-

Table 2. The distribution of results according to the stages for spirometry test and C14 urea breath test in three hundred eighty patients with bronchial asthma (group 1) and chronic obtructive pulmonary disease (group 2).

Groups	Stage 1	Stage 2	Stage 3	Stage 4	p
Tests/Measurements	**Median (Min.-Max.)**	**Median (Min.-Max.)**	**Median (Min.-Max.)**	**Median (Min.-Max.)**	
Group 1					
Spirometry test					
FVC (L,%)	3.02 (2.2-5.7)	2.7 (1.2-4.4)	1.8 (0.9-3.4)	0.9 (0.5-2.1)	<0.001
FEV1 (L,%)	2.6 (1.7-4.4)	2.1 (1.0-3.4)	1.3 (0.7-2.4)	0.7 (0.4-1.3)	<0.001
PEF (L/n,%)	5.6 (2.3-10.2)	4.3 (1.9-8.1)	2.9 (1.0-5.7)	1.9 (0.4-3.8)	<0.001
FEV1/FVC* (%)	85.1± 6.5	78.2± 8.8	72.3 ± 8.2	74.2 ± 13.8	<0.001
C14 urea breath test					
H. pylori load (cpm)	19 (1-408)	97.5 (2-455)	21 (2-577)	17 (1-370)	>0.05
Group 2					
Spirometry test					
FVC (L,%)	2.6 (1.1-4.8)	2.3 (1.4-4.6)	1.7 (0.8-3.4)	1.2 (0.4-2.9)	<0.001
FEV1 (L,%)	2.1 (0.9-4.4)	1.8 (1.1-3.3)	1.2 (0.7-2.7)	0.7 (0.4-2.3)	<0.001
PEF (L/n,%)	5 (3.0-11.9)	4.3 (1.2-8.9)	2.7 (1.1-6.2)	1.4 (0.6-5.2)	<0.001
FEV1/FVC* (%)	84.9 ± 7.6	80.6 ± 8.1	72 ± 10.5	68.1 ± 15.1	<0.001
C14-urea breath test					
H. pylori load (cpm)	19 (4-430)	76 (2-383)	19.5 (1-484)	132.5 (1-541)	<0.05**

**Mean ± standard deviation was used; **The statistically significant difference was due to the stage 4 patients.*
FVC: Forced vital capacity. FEV1: Forced expiratory volume in 1 second. PEF: Peak expiratory flow.

ence ($p>0.05$) between mild asthmatic and control subjects, but seropositivity was significantly higher in patients with peptic ulcer than in the asthmatic patients ($p<0.01$). They advised that these results should be confirmed by further studies. In our series, we found that bacterial load for Hp infection did not differ significantly among the stages of obstructive pattern in patients with BA, in accordance with results reported previously. We concluded that presence of Hp infection in asthmatic patients had no effect on the severity of obstruction.

Chronic obstructive pulmonary disease is a respiratory disease characterized by partially reversible chronic airflow obstruction caused by abnormal inflammation of the lungs, developing from the inhalation of noxious particles or gases. Previous studies reported some relation between seroprevalence of Hp infection and related antibodies. Caselli et al. (17) showed an increase in Hp seroprevalence in the bronchitis group (81.6%) compared with the control group (57.9%). Rosenstock et al. (18) suggested that seropositivity rates in women with COPD might be higher in infected than in uninfected patients. Roussos et al. (19) showed that anti-Hp IgG and CagA levels were significantly different in COPD patients compared to control subjects (53.9% vs. 29.3%). However, they did not determine any difference in pulmonary function test parameters between infected and uninfected patients. Gencer et al. (15) found a significant difference between COPD patients and healthy control subjects in terms of serum anti-Hp IgG levels and Hp seroprevalence. In addition, they revealed that there may be a relationship between serum anti-Hp IgG levels and severity of COPD. In our study, we found a significant difference in very severe COPD patients (stage 4) when C14-UBT mean values were compared with the other stages of COPD and with stage 4 patients with BA. However, when we considered the maximal bacterial load levels, it was also observed that there was a linear correlation between bacterial load and high stages of the disease.

Chronic obstructive pulmonary disease was shown long ago to be associated with peptic ulcer disease, and a decrease in FEV1 and vital capacity was found in smoker and non-smoker gastric ulcer patients (20, 21). Although COPD predominantly affects the pulmonary system, it has numerous extra-pulmonary manifestations (26). Patients with COPD have higher serum levels of several circulating inflammatory markers. It is believed that inflammatory mediators (interleukin [IL]-8, IL-1, tumor necrosis factor [TNF]-alpha, granulocyte-macrophage colony-stimulating factor [GM-CSF], etc.) enhance systemic inflammation, protease/anti-protease imbalance and oxidative stress (7, 22, 23-25). The mechanism of these effects remains unexplained. While this relationship between COPD and peptic ulcer was thought initially to be due to smoking, it was concluded later that Hp infection, rather than the minor effect of tobacco consumption, played the basic role in the pathogenesis of peptic ulcer. Smoking is a well-known risk factor for COPD but is a confounding factor for Hp infection because of variable results in smokers (27-30). Older age, male sex and low socioeconomic status are well-established risk factors for both Hp infection and COPD (10, 31). In this study, the mean age of the COPD group was higher than that of the BA group. This finding can be considered a reason for the significantly higher C14-UBT values that was detected in stage 4 COPD patients. Nevertheless, when we evaluated the age distribution according to other stages in COPD patients (stage 1: 60.3±8.8, stage 2: 58.3± 8.5 stage 3: 59.3± 8.2, and stage 4: 62.7± 9.6 years), no significant difference to support this interpretation was found. In these patient groups, the results for gender, mean age and socioeconomic status during childhood were well-matched within the same stages. The spirometry test and C14-urea breath test results and their statistical significance according to the stages of the disease in 380 patients with bronchial asthma (Group 1) or chronic obstructive pulmonary disease (Group 2). However, Hp infection has been associated with various extra-gastroduodenal pathologies, including respiratory system disorders. Hp infection might be an effective factor in the pathogenesis of COPD by releasing induced secondary inflammatory mediators such as IL-8 and TNF-alpha (32,33). This condition may trigger the endothelial adhesion and migration of inflammatory cells towards the lungs. Therefore, the synergistic effect of common pathways of the cytokine-mediated inflammatory mediators should be considered in the etiopathogenesis of these two disorders, especially based on the

central role of TNF-alpha. In addition, previously shown high anti-Hp CagA strains in infected individuals support this hypothesis in part. We think that the increased bacterial load from Hp infection may contribute to the worsening of pulmonary functions by secondary mediators in patients with high–stage disease (stage 4), under the influence of an increased burden of chronic inflammation.

Conclusion

While no relation was found between Hp load and severity of obstruction in patients with BA or in patients with other stages of COPD, a positive relationship was observed among increased bacterial load and high-stage (stage 4) obstruction in COPD patients.

Declaration of interest

No direct or indirect commercial financial incentive was associated with the publication of this article. All named authors hereby declare that they have no conflicts of interest to disclose.

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Prevalence of Salmonella serotypes in food and water in Belgrade area

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Abstract

Purpose: Foodborne illness caused by *Salmonella* spp. is a worldwide problem. This study reports the results of a two years *Salmonella* surveillance of different sources in Belgrade area.

Methods: A total of 750 samples, samples of water for irrigation and work surface samples were collected and screened for the presence of *Salmonella* serotypes. Detection of *Salmonella* spp. was carried out according to International standard EN ISO 6579:2002 ISO 6579.

Results: *S.* Enteritidis was the most common isolated serotype (56.0%), followed by *S.* group B (12.0%), *S.* Stanleyville (5.3%), *S.* Infantis and *S.* group D (4.0% each), *S.* group C1 (2.7%) and *S.* Mbandaka (2.7%). The remaining serotypes: Derby, Brandenburg, Meleagridis, Tennessee, Tel Hashomer, group 0E-Krefeld, Hadar, Paratyphi B, Veneziana and Typhimurium were present per 1.3%.

Conclusions: A high prevalence of *Salmonella* spp. found in meat and meat products (41.33%) and products of animal origin (36.0%) indicate that food products can be a source of exposure for consumers to *Salmonella* strains in Belgrade area.

Key words: *Salmonella* spp., food infection, water pathogens, transmission, epidemiology

Introduction

Humans become infected with *Salmonella* primarily through faecal contamination of food products or water. Also, contact with ill animals is another source of human infection, affecting farm families, employees and visitors (Wells et al., 2001). *Salmonella enterica* is one of the most common agents causing human gastroenteritis. So far, more than 2 500 serotypes of *S. enterica* have been identified. *S. enterica* serotypes Typhimurium and Enteritidis have been reported to be the most common causes of human salmonellosis in the USA (Humphrey, 2000). However, a large number of other serotypes were also isolated from humans and nonhuman sources.

In Europe, it has been estimated that 10 to 23% of all human salmonellosis are due to consumption of contaminated pork and its products (Mainar-Jaime et al., 2008). Carcass contamination prior to cutting and in the slaughterhouse environment appeared to be important source of *Salmonella* in transport pork and retail products (Sanguankiat et al., 2010). Also, poultry, eggs, beef, seafood (shrimp, oyster, mussel) are also indicated as a vehicle for *Salmonella* serotypes (Padungtod and Kaneene, 2006; Soltan Dallal et al., 2010; Bakr et al., 2011).

The aim of this study was to investigate the presence of Salmonella in meat, meat products, products of animal origin, vegetable products and water for irrigation in rural area of Belgrade.

Materials and methods

Study designe

This cross-sectional study was performed on samples collected from Belgrade area.

This study was carried out according to Microbiology of food and animal feeding stuffs-Horizontal method for the detection of *Salmonella* spp. International standard EN ISO 6579:2002 ISO 6579.

Sample collection

This study was conducted in laboratory for testing of foods in City Institute for Public Health, Belgrade, Serbia.

A total of 750 samples were collected from different sources duing 2010 and 2011. Samples included: meat and meat products (310), products of animal origin (270), vegetable products (110), water for irrigation (40) and work surface swabs (20).

Isolation and identification procedure

1. Pre-enrichment in non-selective liquid medium:

Buffered peptone water is inoculated at ambient temperature with the test portion, then incubated at 37±1°C for the 18±2h. For certain foodstuffs the use of other pre-enrichment procedures is necessary. For large quantities, the buffered peptone water should be heated to 37±1°C before inoculation with the test portion.

2. Enrichment in selective liquid media:

Rappaport-Vassiliadis medium with soya (RVS broth) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn broth) (*Thermo Scientific, USA*) are inoculated with the culture obtained in 1. The RVS broth is incubated at 41.5°±1°C for 24±3h, and the MKTTn broth at 37±1°C for 24±3h.

3. Plating out and identification:

From the cultures obtained in 2., two selective solid media are inoculated:

- xylose lysine deoxycholate agar (XLD agar) (*Acumedia Manufacturers, USA*)

- any other solid selective medium complementary to XLD agar and especially appropriate for the isolation of lactose-positive *Salmonella* and *Salmonella typhi* and *Salmonella typhimurium* strains; the laboratory may chose which medium to use. The XLD agar is incubated at 37±1°C and examined after 24±3h. The second selective agar is incubated according to the manufacturer's recommendations.

4. Confirmation of identity:

Colonies of presumptive *Salmonella* are subcultured, than plated out as described in 3. and their identity is confirmed by means of appropriate biochemical and serological tests.

Biochemical confirmation

Triple sugar/iron agar (TSI agar), Urea agar, L-Lysine decarboxylation Detection of β-galactosidase, Voges-Proskauer (VP) reaction, indole reaction.

Serological confirmation and serotyping

The detection of the presence of *Salmonella* O-, Vi- and H-antigens is tested by slide agglutination with the appropriate sera, from pure colonies and after auto-agglutinable strains have been eliminated (EN ISO 6579:2002 ISO 6579)

Results

Of 750 investigated samples, *S*. Enteritidis was the most common isolated serotype (56.0%), followed by *S*. group B (12.0%), *S*. Stanleyville (5.3%), *S*. Infantis and *S*. group D (4.0% each), S. group C1 (2.7%) and *S*. Mbandaka (2.7%). The remaining serotypes: Derby, Brandenburg, Meleagridis, Tennessee, Tel Hashomer, group 0E-Krefeld, Hadar, Paratyphi B, Veneziana and Typhimurium were present per 1.3%.

S. Enteritidis was the only serotype found in examined samples of raw minced meat (40), ham (10) and cooked mixed meat (10), but not in sausages, raw hamburgers and roast chickens (Table 1). In samples of raw meat (190), this serotype was the most predominant serotype (63.20%) in comparison with *S*. group C1, *S*. group D, *S*. Infantis, *S*. Hadar, *S*. Veneziana, *S*. Paratyphi B and *S*. Typhimurium (5.26% each). At equal frequencies (50.00%) serotypes group D and Derby were detected in sausages (20), while group C1 and Infantis were found in raw hamburgers (20). All samples of roast chicken were positive for *S*. Stanleyville.

In addition to serotypes Infantis and group 0E-Krefeld (6.67% each) which were found in samples of layer cakes (150), *S*. Enteritidis was the most prevalent serotype (86.66%) detected in these samples. However, *S*. Enteritidis was not dominant serotype (20.00%) in samples of Russian salads (50). This serotype was the only serotype found in samples of mayonnaise (10), ice cream (10) and cakes (40). The only serotype found in a sample of pasta with cheese was group D (Table 2). Among vegetable products, *S*. group B, *S*. Brandenburg and *S*. Mbandaka were recovered from samples of baked beans (40), cooked pea (10) and cabbage salad (10), respectively. In samples of sesame seeds was found per one serotype of *S*. Mbandaka (50.00%) and *S*. Tennessee (50.00%). Samples of green salad (20) were positive for *S*. Enteritidis (50.00%) and *S*. group B (50.00%) (Table 3).

Serotypes Enteritidis and Tel Hashomer were found in samples of water for irrigation (40) and Stanleyville in work surface swabs (20).

Table 1. The prevalence of Salmonella serotypes in row meat, meat products and cooked meat foods

Salmonella serotypes	Raw meat	Raw minced meat	Sausage	Raw hamburger	Ham	Roast chicken	Cooked mixed meat	Total
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
S. Enteritidis	120 (63.20)	40 (100)	0	0	10 (100)	0	10 (100)	**180 (24.00)**
S. Stanleyville	0	0	0	0	0	20 (100)	0	**20 (2.67)**
S. Infantis	10 (5.26)	0	0	10 (50.00)	0	0	0	**20 (2.67)**
S. gr D	10 (5.26)	0	10 (50.00)	0	0	0	0	**20 (2.67)**
S. gr C1	10 (5.26)	0	0	10 (50.00)	0	0	0	**20 (2.67)**
S. Derby	0	0	10 (50.00)	0	0	0	0	**10 (1.33)**
S. Hadar	10 (5.26)	0	0	0	0	0	0	**10 (1.33)**
S. Paratyphi B	10 (5.26)	0	0	0	0	0	0	**10 (1.33)**
S. Veneziana	10 (5.26)	0	0	0	0	0	0	**10 (1.33)**
*S.*Typhimurium	10 (5.26)	0	0	0	0	0	0	**10 (1.33)**
Total No (%)	**190 (25.33)**	**40 (5.33)**	**20 (2.67)**	**20 (2.67)**	**10 (1.33)**	**20 (2.67)**	**10 (1.33)**	**310 (41.33)**

Table 2. Prevalence of Salmonella serotypes in some types of ready- to-eat foods

Salmonella serotypes	Russian salad	Mayonnaise	Pasta with cheese	Ice cream	Layer cake	Cake	Total
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
S. Enteritidis	10 (20.00)	10 (100)	0	10 (100)	130 (86.66)	40 (100)	**200 (26.67)**
S. gr B	40 (80.00)	0	0	0	0	0	**40 (5.33)**
S. Infantis	0	0	0	0	10 (6.67)	0	**10 (1.33)**
S. gr D	0	0	10 (100)	0	0	0	**10 (1.33)**
S. 0E-Krefeld	0	0	0	0	10 (6.67)	0	**10 (1.33)**
Total No (%)	**50 (6.67)**	**10 (1.33)**	**10 (1.33)**	**10 (1.33)**	**150 (20.00)**	**40 (5.33)**	**270 (36.00)**

Table 3. Prevalence of Salmonella serotypes in vegetable products

Salmonella serotypes	Baked beans	Cooked pea	Semi prepared eating	Green salad	Cabbage salad	Sesame seed	Total
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
S. Enteritidis	0	0	0	10 (50.00)	0	0	**10 (1.33)**
S. gr B	40 (100)	0	0	10 (50.00)	0	0	**50 (6.67)**
S. Brandenburg	0	10 (100)	0	0	0	0	**10 (1.33)**
S .Meleagridis	0	0	10 (100)	0	0	0	**10 (1.33)**
S. Mbandaka	0	0	0	0	10 (100)	10 (50.00)	**20 (2.67)**
S. Tennessee	0	0	0	0	0	10 (50.00)	**10 (1.33)**
Total No (%)	**40 (5.33)**	**10 (1.33)**	**10 (1.33)**	**20 (2.67)**	**10 (1.33)**	**20 (2.67)**	**110 (14.67)**

Discussion

Salmonella is a prominent food borne disease-causing bacterium and zoonotic pathogen (Griffith et al., 2006). According to Vidanovic et al. (2008), serotypes *S.* Enteritidis, *S.* Typhimurium, *S.* Paratyphi B, *S.* Infantis and *S.* Senftenberg are the most common serotypes isolated from human sources in Serbia. In recent years, *S.* Mbandaka is found to be among fifteen the most frequently isolated serotypes from humans. However, this serotype is very often recovered from animals, particularly from poultry (Vidanovic et al., 2008).

Meat presents a direct food-poisoning threat when consumed in a raw or undercooked form. But, raw meat may serve as an indirect food-poisoning hazard through cross-contamination of cooked meats and of other foods that are not cooked before consumption. Fresh meat may car-

ry *Salmonella* that caused diseases in the slaughtered animals or may be contaminated by handlers (Frazier and Westhoff, 1988).

In this study 41.33% samples were positive. In the investigation of 300 meat samples, 59 (20%) samples were positive for *Salmonella*. The highest prevalence of *Salmonella* was in sausages (26%), followed by minced meat (20%). Burger patty samples contained fewer positive samples (7%). Among 15 serotypes characterized from meat samples, *S.* Anatum was common in both minced meat samples and burger patties but not in sausage samples. The commonest *Salmonella* serotype from both minced meat and sausage samples was *S.* Newport (Mrema et al., 2006). Much lower occurrence of *Salmonella* in sausages (5%) has been reported by Tavechio et al., 2002.

The results of this study showed that *S.* Enteritidis was the only serotype found in all samples of raw minced meat, while *S.* group D (50%) and *S.* Derby (50%) were found in sausages. At similar percentages of 2.7% and 2.9%, *S.* Derby was present in retail pork and retail beef in Mexico, respectively (Zaidi et al., 2006). Mrema et al. (2006) in Botswana reported a 4.2% and 16.7% (prevalence rate) of *S.* Meleagridis and *S.* Typhimurium in minced meat, respectively and a 12.9% and 6.5% (prevalence rate) of *S.* Meleagridis and *S.* Typhimurium in sausages, respectively. *S.* Enteritidis and *S.* Paratyphi B were detected only in sausages (3.23% each), while *S.* Infantis and *S.* Tennessee were found only in minced meat (4.2% each) (Mrema et al., 2006). In the current study, serotypes *S.* Infantis and *S.* group C1 were detected at equal rates of prevalence (50%) in raw hamburgers. On the other hand, *S.* Paratyphi B (100%) and *S.* Typhimurium (100%) were isolated only from raw meat. Since *S.* Typhi and *S.* Paratyphi B are mostly associated with humans, this suggests that the food handlers also contributed to the contamination of these meat products (Forsythe, 2000).

This work also showed the presence of *S.* Veneziana (5.26%) in a sample of raw meat. In the study of one hundred fifty-three wild boars (shot) for the occurrence of foodborne pathogens, most of the *Salmonella* spp. (12%) strains were of serotype *S.* Enteritidis (75%) followed by serotypes *S.* Stourbridge (13%) and *S.* Veneziana (13%) (Wacheck et al., 2010).

S. Stanleyville was detected in 4.9% of retail poultry in Mexico (Zaidi et al., 2006). Similar finding has been observed in the current study, since this serotype was detected in all samples of roast chicken. In the USA, the second most-reported serotype of *Salmonella* causing human disease is *S.* Enteritidis, which causes nearly as many cases of salmonellosis as *S.* Typhimurium, the most prevalent *Salmonella* serotype (CDC, 2008). The European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) have published their annual report on zoonoses and foodborne outbreaks in the European Union for 2010. The report shows that *Salmonella* cases in humans fell by almost 9% in 2010, marking a decrease for the sixth consecutive year. *Salmonella* prevalence in poultry is also clearly declining at the EU level. According to the report, the likely main reasons for the decrease in human salmonellosis cases are the successful EU *Salmonella* control programmes for reducing the prevalence of the bacteria in poultry populations, particularly in laying hens (EFSA, 2012). *Salmonella*, which usually causes fever, diarrhoea and abdominal cramps, accounted for 99,020 reported human cases in 2010 compared to 108,618 in 2009. *Salmonella* was found most often in chicken and turkey meat (EFSA, 2012). The major food vehicle for *S.* Enteritidis is shell eggs, as 80% of the *S.* Enteritidis outbreaks and approximately 50 000 to 110 000 cases are egg associated in the U.S. each year (Braden, 2006). In 2010, a large outbreak caused by *S.* Enteritidis occurred with more than 1800 reported cases and shell eggs were a likely source of infections (CDC, 2010). Eggs can become contaminated internally either by penetration through the shell or directly during formation in the reproductive tract (Howard et al., 2012). In 2010 other Serbian authors examined 1832 samples from poultry, out of which 114 were positive for *Salmonella*. The most common serotypes in this period were *S.* Enteritidis and *S.* Infantis, which accounted for 3.49% and 2.40%, respectively, followed by *S.* Typhimurium and *S.* Hadar (0.16% each) (Stojanov et al., 2011). In the same year, *S.* Thompson was the most common serotype isolated in Iran from 65 (75% of *Salmonella*-positive isolates) chicken, while *S.* Hadar and *S.* Enteritidis were discovered in 7% and 5.8% of chicken positive isolates, respectively (Soltan Dallal et al., 2010). While in

some countries *S*. Infantis can not be found in the material originating from poultry (Muhammada et al., 2010) or it is present in low frequency (1.9%) (Frye and Fedorka-Cray, 2007), this serotype is the most prevalent in Japan (22.6%) (Kanako et al., 2009). Since 2008, *S*. Infantis has taken precedence and become the most common serotype. It became more ubiquitous than *S*. Enteritidis (Ohad et al., 2010). The testing carried out in 2007, showed that *S*. Infantis is a serotype that is frequently found in the samples originating from poultry in Hungary (Nogrady et al., 2007).

In the present study *S*. Enteritidis (100%) was the only serotype found in products such as ice cream, cakes and mayonnaise. This serotype was present in 86.66% of layer cake samples, while *S*. Infantis and *S*. 0E- Krefeld were found at lower rate (6.67% each). *S*. group B was found more prevalent (80%) in comparison with *S*. Enteritidis (20%) in Russian salad samples. From some studies it is known that cakes, ice cream and other bakery products are vehicles of transmission of *S*. Enteritidis and ingredients made from raw eggs, provide a potential source of contamination (Liu et al, 2006). In the largest common source outbreak of gastroenteritis caused by *S*. Enteritidis, cream cake was implicated as the vehicle of transmission. *S*. Enteritidis was isolated from cases, food samples and food handlers (Solhan et al., 2011).

According to the WHO Global Foodborne Infections Network, *S*. Brandenburg ranked 15th among the isolates from human source in Europe in 2006 (WHO, 2009). During 2007 and 2008, some reports place Brandenburg among the 15 *Salmonella* serotypes most frequently identified from turkeys (3.2%) and from pigs (1.1%), respectively (Ricci et al., 2009). In contrast to these results, this investigation showed that *S*. Brandenburg was detected in samples of cooked pea.

In some regions of Latin America, *S*. Meleagridis was the most common serotype isolated from retail pork and retail beef (15.2% and 27.9%, respectively) (Zaidi et al., 2006). In this study S. Meleagridis was found in all samples of semi prepared eating.

The study of a nationwide outbreak with 51 cases of *Salmonella* Stanley infection, strongly indicated that consumption of alfalfa (*Medicago sativa*) sprouts was probably the source of infection (Werner et al., 2007). The samples of sesame seeds were positive for *S*. Mbandaka (50%) and *S*. Tennessee (50%) in the present study. Sheth et al. (2011) described widespread outbreak of salmonellosis caused by *S*. Tennessee. Environmental contamination in the peanut butter plant likely caused this outbreak, which highlights the risk of salmonellosis from heat-processed foods of nonanimal origin.

Baudart et al. (2000) reported the prevalence of serotypes Agona, Enteritidis, Infantis, Mbandaka, Muenster, Rissen, Typhimurium, Montevideo and Virchow in different natural aquatic systems within a Mediterranean coastal watershed (river, wastewater, and marine coastal areas).

Among the 984 isolates from water, isolated serotypes were *S*. Weltevreden (14.5%), *S*. Anatum (11.5%), *S*. Rissen (9.5%) and *S*. Derby (7.2%) in Thailand (Bangtrakulnonth et al., 2004). However, *S*. Molade (71%) was the most common serotype among the water isolates in some African countries (El Hussein et al., 2010). In this study, in samples of water for irrigation it was possible to identify S. Enteritidis and S. Tel Hashomer. Although isolated from frozen frog legs (Andrews et al., 1977), *S*. Tel Hashomer could be classified as rare, since it had not been reported by the Center for Disease Control (Ryder et al., 1978) as isolate from human sources for the years 1968-1974.

Infected food handlers can transmit *Salmonella* organisms to food ingredients, work surfaces and utensils, if personal and food hygiene practices are insufficiently observed (Todd et al., 2007). Several authors reported the occurrence of *Salmonella* at wholesale markets, importers and distributors, where they isolated *Salmonella* from utensils (2%) and floor swabs samples (4%) (FDA, 2004). In the current study, work surface swabs were positive for S. Stanleyville.

Owing to the potential hazard of some serotypes of *Salmonella* it is clearly necessary to put more emphasis on food hygiene. Therefore surveillance of potential contaminant bacteria is crucial for sustenance of public health.

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Exercise program to quality of life following a stroke: Preliminary study

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Abstract

Background and Aim: To determine the quality of life and the effects of ROM exercises on impairments in stroke survivors.

Setting and Design: 82 with stroke observed in the neurology clinic on a follow-up visit; at least a period of 3 months of follow-up was included.

Materials and methods: 82 stroke patients admitted from patients discharged from the neurology clinic of hospital in Yozgat. Age, gender, side of hemiplegia, level of education, comorbid illness, onset to admission interval of all the patients were recorded. The Bartel index (BI) and SF36-functional outcomes at initially (T0) and final (T1) assessment were used to determine the activity of daily living, functional outcomes stroke patients. For stroke survivors, this is a randomized and controlled trial of 5-week exercise program versus usual care. Baseline, post-treatment daily functioning (ADL) assessed by Barthel index, instrumental activities of daily living, Medical Outcomes Study short-form 36-item questionnaire (SF-36). Data were collected using (a) a questionnaire to determine patients' socio-demographic factors, (b) Short Form-36 (SF-36) and (c) Barthel Index from activity of daily living.

Results: At five weeks, arm-leg-trained subjects had improved functional abilities (median Barthel score 15,6± 4,6 - SF 36 functional outcome 17±7,4). It was detected that 56,5±6,4 min:45, max:76 age mean of the patients, gender male; %45,1 female; %54,9, education levels literate % 36,4 primary school 5 year % 29,3 and 8 year % 39. BI and SF 36 functional outcome at T0- T1 were found to be of significant statistical difference. In addition, these differences were observed in correlation with one another. There was no significant correlation between functional outcomes and age, gender, side of hemiplegia, illness period, hospital stay time, economical status, smoking, comorbidity. The statistical difference was detected between patients with education levels. Whereas there was no correlation between functional outcomes and onset to admission interval, the positive correlation was found between BI and SF36 functional outcomes.

Conclusion: Based on the results of this study, we concluded that the patients who suffered from a stroke showed an average therapeutic response on the BI and SF36-functional outcome after discharged from hospital. After discharged from a hospital, stroke patients utilizing an additional exercise intervention led to more rapid improvement in compliance of physical, social, and role function than usual care in persons who suffered from a stroke. Adherence interventions to promote continued exercise after treatment might be needed to extend these benefits to long term. The major finding of this research is that more therapy after stroke results in better physical function and ADL.

Key words: Stroke, daily living activities, exercise, nursing intervention, rehabilitation, quality of life.

Introduction

Strokes remain the third leading cause of deaths in the world as well as being the leading cause of disabilities. This is also consistent with the situation in Turkey 1.

When the rates of illnesses and death caused by strokes, it was observed that there is an increasing trend 2.

Loss of strenght, functional impairments and disabilities significantly affect the quality of life, as they have an impact on patients' daily lives and activities 3,4.

The quality of life is a complex concept that has physical, emotional and social aspects. It is affected by the changes in the daily activities of a patient that suffers from a stroke 5.

Exercise-based interventions can improve functionality and the quality of life 6.

Theraputic exercises after a stroke are beneficial, but limitations and lack of research are issues that come about when specifics of patients' medical history, methodology and monitoring are taken into account 7.

After a stroke, theraputic applications can be followed in acute, sub-actue and cronic phases, and aerobics exercises, strength training and other physical activities can also be incorporated. Older patients and patients who suffered from a stroke benefit more from functional improvements and the quality of life rather than strength and physical improvements 8,9.

Some research exist nationally and globally on the quality of life over various time spans following a stroke 10-14. A study reported that to inadequate exercise health care professional' in Yozgat 13.

UL (upper limb) impairment and ADL independence predicted perceived physical activity. Management strategies for anxiety and therapy for UL recovery long after stroke onset are likely to benefit perceived HRQOL. Stroke-associated disability has been found to affect the daily living of the individual with stroke over a next time 15.

It was designed to observe the improvements over daily lives and qualities of lives of patients who suffered from a sub-acute stroke and following ROM exercises at home.

After a stroke, the patients were observed for up to three months. The research was conducted through the prospective pre- and post-trial test model.

Methodology

This research was conducted through the prospective pre- and post-test model and in a semi-experimental manner. After receiving the written legal permissions required from Yozgat Province Health Directorate, verbal agreement of all patients covered in the research were sought.

The sample

The research was conducted with 82 patients. The following criteria was followed to determine patients to be included: (1) Being in the 30 to 150 days following a stroke, (2) At least 60 points or more scored according to the Barthel Index, (3) Relation to reasons for stroke, (4) The doctor considering no issues with exercise, (5) Ability of communicate verbally, (6) No issues with communicating and memory functions, (7) No serious cardiac symptoms or other health issues that might limit the exercises.

Instruments

Data collected from the case records included type of stroke, affected area of brain and disease duration. A 3-part survey was used for data collection. The questionnaire included (a) a section related to demographics, (b) Barthell Index (ADL=activity of daily living) (c)SF-36 Quality of Life Scale (Medical Outcomes Study short-form 36-item questionnaire, SF-36). The demography section contained basic information regarding; Individual's assigned number of the sample, age, sex, marital status, level of education, economic status, location of stroke, time spent in hospital and the pre-existence of chronic diseases.

Barthel Indeks

The Barthel index (BI) is the scale of activities of daily living. The BI is one of the most commonly used disability scales for rehabilitation patients. It evaluates the mobility and self-care activities. The Barthel Index was used for the functional evaluation. The index evaluates the physical independence in activities of daily living. The Barthel Index is an index that is detailed, objective, easily applicable and understandable, and exact in assessing all specific stages of activities of daily living. Scores of 0-20 indicate totally dependence, 21-61 indicate advanced dependence, 62-90 indicate moderate dependence, 91-99 indicate slight dependence and 100 indicate total independence. The sensitivity of the index was increased using a five-stage scoring system in the Modified Barthel Index modified by Shah. The Modified Barthel Index was adapted in Turkey and the index has proved to be valid and reliable for patient groups with stroke and spinal cord injury. Its modified form is also used for stroke patients. The scale adaptation alpha coefficient of the index is 0.93 for stroke patients in the Turkish society. The Barthel Index was developed in order to assess the independence of patients, regarding their self care duties. The 10-item BI evaluates the feeding, transfer from chair to bed, grooming, sitting on the toilet, bathing, ambulation, stair climbing, dressing, bladder and bowel con-

trol. The Barthel Index takes some criteria, such as bladder and bowel control, grooming, toilet use, feeding, transfer, mobility, dressing and bathing into consideration 16-17.

SF-36 Quality of Life Index – Short Form

This short form was developed for application by the Rand Corporation to evaluate the quality of life. It was also translated to Turkish, and its applicability and reliability were tested. It utilizes generic criteria for self-determination. It comprises of 36 items measuring towards 8 dimensions. (1) Physical condition: Limitations to physical activities due to health issues (pushing a table, carrying bags, climbing stairs, walking and other such daily activities). (2) Social functions: physical and emotional reasons for limitations (visits of friends and relatives). (3) Role-specific physical limitations: Effects on role-specific functions such as work hours and effects of work on health. (4) Emotional functions: Role limitations (Emotional issues arising from limitations to daily life such as depression. (5) Mental health: Mental health in correlation with physical issues and wellness. (6) Fitness (overall energy, objective evaluation of the level of tiredness). (7) Pain: How strength of pain effects rehabilitation. (8) General Status of Health (self-evaluation). Except for some of these points, Likert type evaluation was adopted and the prior 4 weeks were evaluated. The scale of 0-100 was utilized to determine health, and higher the score, better the health. It was observed that this measure is appropriate for patients suffering from physical issues to measure the quality of life 18,19.

Nursing intervention: The passive and active (possible) ROM exercise program was designed to improve strength, balance and activities of daily living, and to encourage more use of the affected side. The experimental exercise intervention was initiated after discharged of baseline testing. It was a home-based exercise program prepared by a researcher. The study principal investigator (a researcher) observed at least 1 therapy session for each subject to ensure standard application of interventions. The program included 2 visits (T0 initially, T1 end visit), a total of a week for five weeks, and the patients were instructed to continue the exercise program on their own for 4 additional weeks. Each exercise session lasted ≈30 minutes. Exercise sessions were divided into 4 blocks preceded by a 5-minute warm-up session of stretching and flexibility exercise. The first block included assistive and resistive exercises using ROM (range of motion) or elastic bands exercise to the major muscle groups of the upper and lower extremities. The movement patterns included (a) flexion, abduction, and external rotation of shoulder with the elbow extended and with wrist and finger extension; (b) extension, adduction, and internal rotation of shoulder with elbow extended and with finger and wrist flexion; (c) flexion, adduction, external rotation of hips with knee flexion, and ankle dorsiflexion; and (d) extension, abduction, internal rotation of hips with knee extension, and ankle plantar flexion 20. Elastic bands of varying elasticity used as a means to provide resistance. All group subjects took 5 weeks to complete nurse-directed sessions.

The region of Yozgat state hospital did not apply exercise after stroke.

Evaluation of Results

The pre- and post-test values of the sample were recorded and assessed using computer software such as Windows SPSS v. 11. To analyze the data, k-square, multi-variable variance, and independent t-tests were conducted. The results were evaluated using 95% confidence interval and p=0.05 meaningfulness scale.

Ethical Consideration

Permission was obtained from the Yozgat Governorship in order to conduct the study. The purpose of this study was explained to the patients to be included in the study and the required institutional permissions were obtained from Yozgat Provincial Directorate of Health and Yozgat State Hospital for this study. Individuals who accepted to participate in the study were informed about the purpose, expected results and estimated time of the study, and that they may withdraw from the study at any stage. The volunteers were included in the study. These individuals were informed about risks of exercises and medical benefits expected from the study. Those who voluntarily accepted to participate in the study were included in the study.

Limitations and Generalization

The results from the research can be generalized to cover the entire sample. The limitation to this is that the research was conducted over a small sample size.

Results

Eighty-two individuals were studied after a stroke. The results of this study, showed that the patients with stroke showed an average therapeutic response on the BI and SF36-functional outcome after discharged from hospital. The mean scores of the subscales of quality of life are shown in (Table 1). It was detected that 56,5±6,4 min:45, max:76 age mean of the patients, gender male; %45,1 female; %54,9, education levels literate % 36,4 primary school 5 year % 29,3 and 8 year % 39. BI and SF 36 functional outcome at T0- T1 were found significantly statistical difference.

At five weeks, arm-leg-trained subjects had improved functional abilities (median Barthel score 15,6± 4,6 - SF 36 functional outcome 17±7,4). BI and SF 36 functional outcome at T0- T1 were found significantly statistical difference. In addition to, these differences were shown each other correlation. There was no significant correlation between functional outcomes and age, gender, side of hemiplegia, illness period, hospital stay time, economical status, smoking, comorbidity. The statistical difference was detected between patients with education levels. Whereas there was no correlation between functional outcomes and onset to admission interval, the positive correlation was found between BI and SF36 functional outcomes.

At baseline, participants had mild deficits in self-care, and most had deficits in physical limitations and household and community activities. There were significant deficits in most indicators of QOL. Baseline stroke function and QOL were similar between treatment arms-legs, except initial time controls had slightly lower ADL status by BI score.

T0-T1 groups increased the proportion of participants who were independent in basic ADLs (Barthel score) and who were independent in community ambulation. The groups demonstrated

Table 1. SF 36 Quality-of-life Measures (Mean [SD])

Quality-of-life instrument	Min.Max	$\bar{x}$ ±SD
Functional capacity	8-82	48.43±12.27
Well-being	10-89	46.08±22.77
General health perception	3-90	44.72±26.56
Global quality of life	13-80	37.08±14.03

Table 2. Stroke patients education levels correlation BI difference scores

Education Levels (year)	BI difference point scores			
	10*	15	20+**	Total
Literate	10	13	6	29
5 year	6	15	11	32
8 year	0	10	11	21
Total	**16**	**38**	**28**	**82**

** Illiterate 5 person include literate*
*** 25 point scores 3 person*

Table 3. Stroke patients BI and SF36 functional outcome

Tests	* $\bar{x}$ ± S	Paired samples test	Paired samples correlation
BI pre-test	63,9 ± 3,8		r= 0,55 p<0,001
BI post-test	79,5 ± 5,4		
BI difference	15,6 ± 4,6	t =30,6 p<0,001	
SF36 functional pre-test	36,4 ± 10,7		r= 0,80 p<0,001
SF36 functional post-test	53,4 ± 12,6		
SF36 functional difference	17,0 ± 7,4	t =15,3 p<0,001	

BI difference with SF36 functional outcome difference scores relatively relationship r= 0,22 p<0,05

increasing scores in SF-36, and BI scales. Interventions were more effective on SF-36 physical function, Barthel score. There were no detectable effects on the motor or cognition score.

Discussion

This was a semi-experimental and randomized, controlled trial of accelerated hospital discharge and home-based stroke rehabilitation examined. Individuals with non-communicable diseases experience long-term physical and psychosocial limitations, and can lose their independence. Even patients with no serious physical handicap may still confront many difficulties in daily life, which can contribute to dissatisfaction 21.

Our study, after five weeks outcome measured support the notion that activities of daily living to a rehabilitation course in the community has no adverse effect on outcome. The Barthel score is the most widely used measure in stroke rehabilitation trials and was used in this study because of the importance of shorter term disability. Patients may expect to be cared for by nurses and not to be encouraged to independence in the hospital, while at home the need for independence may be more evident 22.

The overall survival rates were similar in both groups, and early discharge did not result in greater rates of institutionalization or hospital readmission. Although the total therapy for each discipline provided was not significantly different between the two groups for those having therapy, provision of therapy for patients with an impairment was better in the patients treated in the community, possibly reflecting better assessment and team work.

In this study, the quality of life of patients with ischemic stroke was found to be insufficient, which is similar to the observations made in earlier studies 23,24.

Post discharge additional exercise variegated for suffer from stroke can give better results also because of a plasebo effect. The results from previous trial, aimed to verify our initial Duncan et al. 1998 (25) "more is better", were difficult to interpret because randomization (single group) cultural and evaluation of patients was not blind. We tried to overcome these difficulties by randomization including in our study only patients with mild disability due to ischemic stroke. Our study results show that, extra exercise intervention mild severity ischemic stroke patients were lead to improvement when compared with a conventional care.

Comparison with other studies

Evidence of effectiveness of home-based stroke rehabilitation is available from several randomized controlled trials conducted. Almost have concluded that home-based rehabilitation after stroke is feasible, acceptable to patients (and caregivers), and as effective as routine care and rehabilitation 26.

Little attention has been has been focused on the literature to the rigorous examination of the therapeutic aspect of the nursing role in stroke rehabilitation 27,28. Early discharge schemes have been developed for stroke patients and caregivers 29. Experimental patients did receive additional nursing care after discharge, although the therapeutic focus of that care is not disclosed 26,30. Supported discharge and rehabilitation at home Therapeutic nursing content of home-based rehabilitation package not specified. Augmented support hospital discharge and follow-up at home reduce readmissions to hospital of specially elderly patients, thereby saving hospital resources.31

Conclusion

This study might have been vague about difference the between the outcome of the two rehabilitative approaches. Firstly, patients were enrolled in different intensity. It may be that a group of less severely affected patients would have benefited. Secondly, the patients received the extra-therapy for only five weeks, and this may be a too short term. Thirdly, single group pre-test post-test study. However, caution is needed in interpreting this result, because the sample size is small. In conclusion, accelerated hospital discharge and home-based ROM exercises proved to be a practical and effective alternative to conventional care and improves quality of life for patients with stroke, and resulted in a significant increase in BI. This rising in BI may make home-based exercises an attractive and cost-effective means of rehabilitative intervention some patients with stroke in the Yozgat setting. However, further detailed investigation of the cost implications of such rehabilitation and exercises schemes is needed before they can be adopted unreservedly.

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Why change the treatment of diabetic patients?

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Abstract

Purpose: The aim of our study is to examine the reasons of the transition to insulin treatment in type 2 diabetes patients who are insulin users and under control in our diabetes polyclinic.

Method: The study was made with 305 patients diagnosed as type 2 diabetes mellitus who were under control in the diabetes polyclinic of Haseki Training and Research Hospital and took insulin treatment between 2008-2009. The files of the patients were studied retrospectively and the reasons of transition to insulin were investigated.

Results: Of the 305 type 2 diabetes mellitus patients in our study, 157 (48 %) patients were male, 148 (52 %) patients were female, 249 (81.6 %) patients were between the ages of 18 and 65, 56 (18.4 %) patients were above 65 years old. It was found out that the most common reason of the transition to insulin is the lack of oral anti-diabetic treatment (OAD) with the rate of 41.81 %.

Conclusion: Insulin can be started in any period of diabates in order to decrease the development risk of acute and chronic, micro and macro-vascular complications which the disease causes. The progress of the disease, the importance of the treatment and the outcomes of the disease should be told to the patients in every period in order to break the resistance which the patients create against the treatment.

Key words: Diabetes mellitus, insulin treatment, insulin treatment indications.

Introduction

Type 2 diabates mellitus (DM) is a progressive disease which causes losing pancreas cells in the following years. According to the findings of The United Kingdom Prospective Diabetes Study (UKPDS), it is known that only 50 % of the cells have a function in the process of diagnosis in the patients diagnosed as type 2 diabetes mellitus. When looking at the patients who change their life styles and who are treated with biguanid, sulphonilure or insulin after six years following the treatment, it is seen that the functional cell rate is only 25 %. Approxiametely 10-15 years after the diagnosis of type 2 DM, the secretion of the endogenous insulin is 10 % of the normal level and exogenous insulin treatment is needed. Because of that, including medicine which protects production of pancreas cell mass and insulin may decrease the insulin resistance; it may slow down the progress of exogenous insulin treatment, it may stop it or take it backwards (1-4). Doing exercises, a diet and if needed a medicine treatment should be given to the diabetic patients for efficient glysemic and metabolic control and good patient training should accompany with each of these steps (5,6). The findings acquired from studies carried out on both type 1 and type 2 diabetes patients show that early extensive hyperglisemic follow-up and treatments have quite outstanding and useful outcomes in order to decrease the microvascular complications in a long period (7). The most important point in order to prevent or postpone chronic complications, to make diabetes patients' life qualities better and to decrease the health expenses is efficient glysemic control (8). If patients cannot reach the target hemoglobin A1c level although they change their life styles and take anti-diabetic, drugs, under this circumstance, possibly, the most important agent insulin should be used. Insulin treatment should be considered as a start for the patients who have a serious hyperglycemia (hunger plasma glucose is APG>350 mg/dL) and a ketonuria, or for the ones who cannot tolerate oral anti-diabetic (OAD) agents or have contraindications against these agents (9). In fact, many patients who have started taking insulin treatment nowadays have had type 2 diabetes (correspondingly developed complications) for 10-15 years

(10). Since secretion of endogenous insulin gets lower in time, type 2 diabetes patients will need insulin at the end. Some authorities, although not common in the present application, support an agressive treatment which will include insulin earlier (11,12). Fears and obstacles against using insulin have decreased considerably with the production of insulin analogs and ready mixtures and with the fact that more practical devices were developed for insulin injection (13).

The aim of our study is to examine the reasons of the transition to insulin treatment in type 2 diabetes patients who are insulin users and under the control in our diabetes polyclinic. The importance of treatment planning is emphasized in order to prevent micro and macro complications and make patients have more qualified lives by enabling better glysemic levels.

Methods

The study was made with 305 patients diagnosed as type 2 diabetes mellitus who were under control in the diabetes polyclinic of Haseki Training and Research Hospital and took insulin treatment between 2008-2009. The study was approved by the ethic committee moderated by Prof.Dr. Ayten Altintaş on the date 08.12.2009 with the decision number 1. In the process of diabetes diagnosis, the guide of diagnosis, treatment and observation for diabetes mellitus of Turkey Endocrinology and Metabolism Association (TEMD) was taken as a base (14). According to this guide, a diabetes mellitus patient (DM) possibly whose random glucose value (+diabetes complications) is ≥200 mg/dl or a hunger plasma glucose (following at least an eight hour hunger) is ≥126 mg/dl or 2nd hour plasma glucose value in the oral glucose tolerancee test (OGTT) is (75 g glukoz) ≥200 mg/dl was diagnosed as distorted glucose tolerance (IGT) if his 2nd hour plasma glucose in OGTT is 140-199 mg/dl, and was diagnosed as distorted hunger glucose (IFG) if hunger plasma glucose is 100-125 mg/dl. According to the criteria of World Health Organisation, gestational DM criteria were defined as 75 g glucose and APG≥126 mg/dl in OGTT or 2nd hour plasma glucose is≥200 mg/dl. On the other hand, American Diabetes Academy has determined on the GDM criteria as 75 g glucose and APG≥95 in OGTT (it requires at least two pathological value diagnosis); in the 1st hour ≥180, in the 2nd hour ≥155 mg/dl or 100 g glucose and APG≥95 in OGTT (it requries at least two pathological value dignosis); in the 1st hour ≥180, in the 2nd hour ≥155 and in the 3rd hour ≥140 mg/dl (15).

The files of the patients were studied retrospectively and the reasons of transition to insulin were investigated. In the cases, UKPDS data and the TEMD 2009 guide of diagnosis, treatment and observation for diabates mellitus and its complications were taken as a base. In the light of these guides, the cases were investigated for a good metabolic control inability with OAD, losing too much weight, hyperglysemic symptoms, macrovascular complication (coroner artery diseae, stroke, peripheric artery disease), microvascular complication (neuropathy, retinopathy, nephropathy), systemic diseases, acute complication (hyperosmos, hyperglysemic situation-HHD or diabetic ketoacidosis-DKA), major operation, pregnancy and lactation, kidney or liver deficiency. These parameters were compared by asking the patients' diabetic years, ages and their defence which they show to the doctors for insulin treatment.

In the course of evaluating the data statistically, Statistical Package for Social Sciences for Windows (SPSS) 16.0 programme was used. Numerical values were evaluated as average±standard deviation and categorical data were evaluated as frequency/percent. Whether the distribution of the values are homegenious for each group or not was tested with Kolmogorov-Smirnov Z test. If the distribution was normal with the numerical data, student T test was used for binary comparison. In the contrary situation, Mann-Whitney U test was used. While evaluating categorical variables, Chi-squared test was used and confidence interval $p<0.05$ or 95% were accepted as statistically meaningful.

Results

Of the 305 type 2 diabetes mellitus patients in our study, 157 (48 %) patients were male, 148 (52 %) patients were female, 249 (81.6 %) patients were between the ages of 18 and 65, 56 (18.4 %) patients were above 65 years old. Oral anti-diabetic treatment deficiency with the 41.81 % of the patients, chronic complications (nephropathy, reti-

nopathy, neuropathy) with 14.17 % of the patients, acute complications of diabetes (DKA, HHD) with the 7.42 % of the patients , major operation with the 10.25 % of the patients, losing too much weight with the 1.61 % of the patients, systemic diseases (KKY, malignities) with the 8.24 % of the patients, macrovascular complication (ischemic heart disease) with the 6.23 % of the patients, pregnancy with the 1.31 % of the patients , hyperglysemic symptoms with the 0.30 % of the patients resulted in transtion to insulin treatment. Of all these parameters, the most common reason is the inability of controling glysemia efficiently in OADs. (Figure 1)

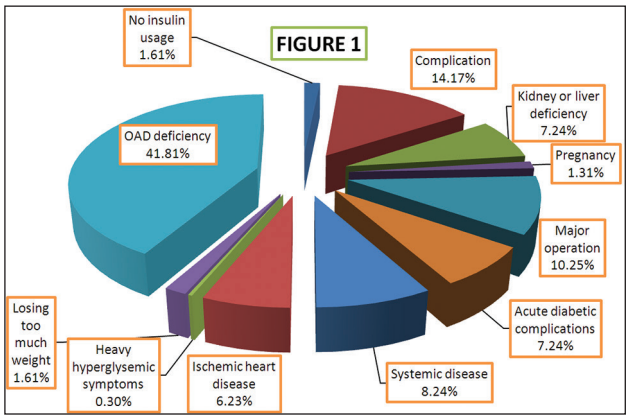

Figure 1. The indications of transition to insulin for type 2 diabetes patients

Comparing the reasons of transition to insulin with gender, except from major operations and acute diabetic complications, there was no statistically meaningful difference. Although there was no meaningful difference, it was observed that OAD deficiency is a more common reason in women (F: 52%, M: 48%; p=0.305). It was found out that women develop more chronic complications (F: 55.8%, M: 44.2%; p=0.591). It was seen that kidney and/or liver deficiency is a more reason for transition to insulin in men(M: 54.5%, F: 45.5%; p=0.557). Similarly, it was discovered that most of the patients who start to use insulin as a result of a major operation are males (M: 67.7%, F: 32.3%; p=0.045). It was found out that women start insulin more than men because of acute complications (F: 77.3%, M: 22.7%; p=0.012). Although there is no big difference between the genders, it was seen that men start insulin a bit more often than women as a result of a systemic disease (M: 52%, F: 48%; p=0.065). And again, it was found out that men start insulin more often that women because of macrovascular complications (M: 52.6%, F: 47.4%; p=0.413). Transition to insulin as a result of a heavy hyperglysemic symptoms was only seen with women (p=0.321). Men were seen more often to start insulin as a result of losing too much weight (M: 60%, F: 40%; p=0.369). 88 % of our patients (268 patients) in our study started insulin treatment without showing defence against doctors and 12 % of them (37 patients) started insulin with showing defence. Of the patients whose ages are above 65, 17.9 % with defence, 82.1 % without defence; of the patients whose ages are between 18-65, 10.8 % with defence, 89.2 % without defence started insulin treatment. It was found out that 15.3 % of women and 8.8 % of men started insulin treatment with showing defence against doctors (p=0.082). Considering age and gender together, 17.9 % of the 18-65 age group male patients showed defence to the doctor and 82.1 % did not. 10.2 % female patients showed defence and 89.2 % did not (p=0.146). Whereas 17.5 % of the female patients whose ages are above 65 showed defence to the doctor, 82.5 % did not. With regard to the male patients, 10.9 % showed defence to the doctor and 89.1 did not (p=0.165). As the patients are older, the defence they show gets more, but it was seen that there is no distinct difference between the genders.

It was noted that 133 patients for 0-10 years, 141 patients for 10-20 years, 25 patients 20-30 years nad 6 patients for 30 years were observed with the diagnosis of diabetes. Although the diabetic ages of the patients are high, 10.8 % of the patients showed defence to the doctor and 14.54 % patients did not. The finding that as the diabetic year is higher, the defence against the doctor gets more was found meaningful.

Discussion

The frequency of diabates has been increasing in the whole world. It is declared that the number of the diabetes patients which was probably 171 million in 2000s will reach 366 million in 2030s (1). Treatment approaches towards preventing diabetes and controlling efficiently are needed because of its epidemic dimension and socioeconomic burden on society. Although transtion into a life style

which is based on appropriate feeding and increasing physical activity (LSC) is a requirement for treating diabetes, adding medicine treatment to the process is a necessity most of the time in order to reaach the target values. OADs are effective on one or more pathophysiological disorders in diabetes in recent time (16). It is stated that age, gender, socioeconomic position, education level, type of diabetes and its duration are variables which may influence comprehending the seriousness of the disease, its treatment and outcomes (6,17-21).

The importance of gender was investigated in approaches and applications to diabetes through some researches and different results were gained. It was seen in Coates and Boores' studies that women consider diabetes less risky that men do (22). It was found that there is a little difference between genders when focusing on approaches towards diabetes and adaptation to the treatment in Fitzgerald's and his collegues' study(23). In our study, 48.5 % were male and 51.5 % were female of 305 patients. The rate of the patients who were given insulin treatment in both male and female patients were found close and there could not be found a meaningful difference.

It was noted in literatüre that young patients are anxious about their care and treatment, they develop social and medical anxieties with the fear of developing complications as they grow older and that the way they comprehend the disease changes (24). It was found out that 56 (18.4%) of the patients involved in our study who started insulin were above 65 years old and 249 (81.6 %) were between the ages 18 and 65. Age, gender and diabetic year are effective factors on the defence in the process of transition to insulin. 17.9 % of the patients above 65 started to take insulin with showing defence to the doctor and 82.1 % did not show any defence; 10.8 % of the patients whose ages are between 18 and 65 started to take insulin with showing defence to the doctor and 89.2% did not show any defence. While the defence to the doctor gets lower as the age and the diabetic year gets higher, insulin treatment which advised by the doctor is rejected in the young ages and in the early periods of diabetes because of getting wrong information and the fear of insulin shot. This situation enhances the risk of all possible complications and decreases the quality of the patient's life.

It was seen that 15.3 % of the women and 8.8 % of the men started insulin treatment with showing defence to the doctor (p=0.082). Besides, it was noted that there is no big difference between gender on the point of showing defence to the doctor. These data indicate that more aggresive treatment is necessary for diabetes and the way of treatment should be changed at once on the condition that the patient cannot reach the glysemic targets. When glysemic control is enabled, patients usually feel better and energetic. United Kingdom Prospective Diabetes Study (UKPDS) shows that more than the half of the diabates patients population need insulin at the end and most of these patients are presumed to need insulin for the rest of their lives (2). The patients who are started insulin treatment at the present time have already had type 2 diabetes (consequently developed complications) for 10-15 years (10). Of all the type 2 diabetes patients in our study, 133 patients for 0-10 years, 141 patients for 10-20 years, 25 patients for 20-30 years, 6 patients for 30 years have been under control with the diagnosis of diabetes. Although diabetic years of the patients are high, in the rate of 10.8 patients shoed defence to the doctor and 14.54 did not. So, it was found that showing defence gets higher in a meaningful rate as the diabetic year gets higher through this comparison. Since this defence makes uncontrolled glysemic period longer, it causes development of acute, chronic, micro and macro complications. There could not be found any meaningful result between patients' ages, genders and showing defence to the doctor. However, it was found out that all of the patients who have ischemic heart disease and diabetic chronic complications are men.

Oral anti-diabetics may be ineffective and may not be tolerated by some type 2 diabetes patients. Under this condition, insulin treatment should be started as soon as the diagnosis is done. It is especially important with the patients who have hyperglysemia and serious problems together. In our study, 41.8 % of the patients were started insulin treatment since glysemic control could not achieved with OADs. This parameter was regarded as the most common reason in the process of transition to insulin treatment. Of all these patients, 52 % were female and 48 % were male. When its relation was compared with gender, it was observed

that it is more often to come across inability of controling glysemic level efficiently with OADs. 0.3 % of the patients because of the development of heavy hyperglysemic symptoms; 8.2 % of them because of the other available systemic diseases (KKY, malignities); 10.2 % of the patients after the major operations (by-pass, canser operations) and 1.6 % of the patients started insulin since they lost too much weight. A meaningful difference could not be found when the relation was analysed between the patients who started heavy hyperglysemic sypmtoms, acute complications, systemic disease, major operation and gender.

In some studies such as The Diabetes Control and Complication Trial (DCCT), it was found out that high HbA1c causes development of nephropathy and intensive insulin treatment is effective both in starting period and obvious nephropathy period for type 1 diabetes (7, 25-27). The difference between the control group and intensive treatment in the period of nephropathy was explained in UKPDS which was made with type diabetes mellitus patients (25-28). In our study, 14.17 % of the patients started insulin because of the chronic diabetic complications and 7.24 % started insulin acute complications of diabetes. According to our research, chronic complications were observed more than acute complications and it was seen more often with women.

In the study of Naka and his collegues, it was noted that the impetus of the diabetes is influencial on the frequency of myocardial infarctus, but since HbA1c used to detect it shows a short term glysemic control, it is an insufficient parameter (29). In our study, it was found out that 6.23 % of the patients started ischemic heart disease. It was remarked that the insulin resistance which type 2 diabetes mellitus patients and obese have causes hepatic steatosis and furthermore it has a basic role for the physology of the non-alcoholic fatty liver diseases (30). In our study, it was seen that 7.24 % of the patients started insulin treatment as a result of liver and/or renal deficiency. It was concluded that as the need for insulin gets more, the possibility of liver and renal deficiency development increases. According to TEMD's suggestion, in gestational diabetes mellitus, GDM research should be done for all of the pregnant women (whether they have a risk or not) in Turkey in the 24th-28th weeks of the pregnancy in order to decrease the macrosomia in fetüs and related risks, to protect the prospective mother and in order to control risky women who have a potential for type 2 diabets and insulin resistance. Considering the treatment, we need more data for using OADs in GDM. Therefore, insulin should be used for the GDM cases which cannot be controlled with diet and exercises. In our study, 1.3 % of the patients started insulin because of pregnancy.

Consequently, the same result with the international researches was found in our study that insufficient control of OAD is the most common reason of transition to insulin treatment. The effect of gender in this process was studied and it was noted that there is no big difference between genders. Macro and micro complications of diabetes are other reasons for transition to diabetes. It was concluded that as the need for insulin gets more, the possivlity of liver and renal deficiency inreases. It was announced in literatüre that young patients are anxious about their care and treatment, they develop social and medical anxieties with the fear of developing complications as they grow older and that the way they comprehend the disease changes. It was seen that as the patients get older, they show less defence to the doctor. However, as the diabetic age gets higher, showing defence gets higher. That causes inability of controling glysemia. Besides, the risk of complication development increases.

Training diabetes patients is one of the corner stones of the treatment. Some factors such as diabetic year, health security and education level should be considered while preparing clinical treatment programme for the diabetes patients and they should be informed about diabetes (6).

The process:

Insulin treatment should be started for type 2 diabetes patients if treatment targets cannot be reached with OADs.

Glysemic control targets and insulin doses should be personalized.

The choice between insulin analogs or ready mixture insulin analogs should based on factors such as practicality, flexibility, simplicity and what is needed, moreover OAD usage, general glysemic control and complication and empitus of the disease.

Basal-bolus treatment based on analog insulin which is fast efficient with meals, long efficient

for basal need is more effective on the patients whose functional disorder in their beta cells are in advanced level and it gives not only a flexible life style but also an intensive insulin regime.

In the place where conditions are appropriate, a patient should work with his diabetes team and play an active role to manahe his own disease.

Conclusion

Type 2 diabetes is a chronicand progressive disease. Insulin can be started in any period of the disease in order to decrease the risk of acute and chronic, micro and macrovascular complications which the disease causes. The progress of the disease, the importance of the treatment and the outcomes of the disease should be told to the patients in every period in order to break the resistance which the patients create against the treatment. The patients should work with a diabetes team and should have an active role in order to manage his own disease.

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The nutrition and health status of adolescents living in Turkish orphanages

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Abstract

Objective: This research has been conducted in order to examine the nutrition and health situations of adolescents staying in orphanages in Ankara/Turkey.

Methods: A total of 198 adolescents consisting of 115 male and 83 females between the ages of 13-18 make up the target population of the study. The demographic characteristics, nutrition habits by using a healthy eating index (HEI) scale, anthropometric measurements and biochemical findings of adolescents have been evaluated in this study.

Results: HEI score of 15.7% of the males has been evaluated as "bad", 80.9% "medium" and only 3.5% has been evaluated as "good". Anthropometric measurements, average diastolic-systolic blood pressure, serum glucose, Blood Urea Nitrogen (BUN), Alanin Amino Transferaz (ALT), Aspartat Amino Transferaz (AST), calcium, cholesterol, triglyceride, uric acid, protein, albumen, zinc, iron, iron binding capacity, Vit B_{12} levels of the adolescents have been found within the reference values of adolescents between the ages of 13-18. The serum glucose, BUN, calcium, protein, albumen, iron binding capacity ($p<0.001$) and Vit B_{12} ($p<0.005$) levels according to gender has demonstrated major differences. The serum glucose, BUN, protein, albumen, and zinc, Vit B_{12} level averages of males compared to females; and the average serum calcium and iron binding capacity levels of females compared to males has been determined to be higher.

Conclusion: It has been suggested to develop and apply an education program to improve the diet habits, strengthen the nutrition and health information of adolescents; and to have all orphans staying in orphanages to adapt a healthy lifestyle.

Keywords: Adolescence, nutrition, HEI, anthropometric measurement, orphanages.

Introduction

Family life consistently seen to be correlated with more positive and fewer negative outcomes for young people is eating meals together as a family, and there have been concerns about whether family meals are becoming less frequent in modern times. Some extant research finds that 25–30 % of adolescent respondents report dining with their families 7 days a week, but similar or higher percentages report dining with their families 2 or fewer days a week. Frequent family meals are associated with better nutritional intake and better school performances.[1-3] Family carries the nutritional habits and preferences presenting long term affects. The diets of adolescents who regularly eat with their families are better in terms of nutritional variety and quality.[4] None the less, family meals in our day and age are more seldom due to factors like the number of people in the family, changes in the family structure, parents living apart from each other or working mothers. This situation has a negative effect on the food choices of adolescents.[5] It is known that adolescents frequently use nutrition to indicate their discontent against family authority. These attitudes consist of overeating, choosing food, diets as a passing fancy and skipping meals. For this reason, parents must aim being a model for their children.[6, 7] Although there are studies researching the nutrition situations and habits of adolescents, and the effects of these on health, the number of studies on adolescents who have to live in orphanages and who are trying to prepare for life on their own without the concept of a guiding family is rather low despite the increasing importance of the subject. This study is important in terms of presenting new suggestions related to the nutrition and health situations of adolescents living in orphanages and in terms of determining the relationship of nutrition and physical activity situations of 13-18 age group adolescents with health and body composition.

Methods

Participants

All adolescents (n=198) between the ages of 13-18 living in the five orphanages of Prime Ministry Social Service and Children Protection Agency General Directorate willing to participate in the study and who are not mentally disabled have been included in the study. Data were collected through face to face interviews in a 6-month period. The study protocol was approved by the directorate of Orphanages and by the General Management of Prime Ministry Social Services and Child Protection Agency, by the Gazi University Scientific Research and Ethical Council of Faculty of Medicine. Those adolescents who were 13-18 years of age signed consent forms to participate in this study and directorate of Orphanages authenticate of the study protocol.

The questionnaire

The questionnaire contained socio-demographic items including sex, age, educational and family status. Data were collected in a personal interview.

Healthy eating index (HEI) scores

As the healthy eating index (HEI) was originally developed as a measure of diet quality in the US, it was modified for Turkish youth. The HEI-2005 is a valid measure of diet quality. Potential uses include population monitoring, evaluation of interventions, and research.[8] HEI scores were derived from previous-day dietary intake data collected as part of the Mobile Examination Center interview/examination via a trained interviewer/dietitian-administered 24-hour recall. HEI scores range from 0 to 100, with 10 equally weighted components, each with a score ranging from 0 to 10. The first five components of the HEI measure the degree to which a person's diet confirms to the dietary guidelines for Turkish people prepared by Turkish Ministry of Health and Hacettepe University Department of Nutrition and Dietetics in 2004. Food and Nutrition Council of United States of America stated that foods should be classified under four groups in 1958. This classification facilitates making daily food plan. Council has passed "Food Pyramid" usage in 1985 with the view of contributing consumer consciousness in nutrition. Countries make changes in pyramid according to their food habits and food availability. Clover with four leaves has been used for Turkey in expression of groups with shape since it is appropriate using four food groups in planning daily food intake.[9]

The maximum score of 10 indicates that the recommended servings were reached, while a zero indicates that no foods in that group were

Table 1. Criteria for minimum and maximum scores for each HEI[a] component[b]

Component	Dietary guidelines for Turkey[e]		Dietary guidelines for USA[f]	
	Criteria for minimum score (0)	Criteria for maximum score (10)[d]	Criteria for minimum score (0)	Criteria for maximum score (10)[d]
Grain consumption	0 servings[e]	6-11 servings[e]	0 servings[f]	6-11 servings[f]
Vegetable consumption	0 servings[e]	3-5 servings[e]	0 servings[f]	3-5 servings[f]
Fruit consumption	0 servings[e]	2-4 servings[e]	0 servings[f]	2-4 servings[f]
Milk consumption	0 servings[e]	2 servings[e]	0 servings[f]	3-4 servings[f]
Meat consumption	0 servings[e]	2 servings[e]	0 servings[f]	2-3 servings[f]
Total fat intake	≥45% of kcal[f]	≤35% of kcal[e]	≥45% of kcal[f]	≤30% of kcal[f]
Saturated fat intake	≥15% of kcal[f]	<8 % of kcal[e]	≥15% of kcal[f]	< 10% of kcal[f]
Cholesterol intake	≥ 450 mg[f]	≤ 300 mg[e]	≥ 450 mg[f]	≤ 300 mg[f]
Sodium intake	≥ 4800 mg[f]	≤ 2400 mg[e]	≥ 4800 mg[f]	≤ 2400 mg[f]
Food variety	≤ 3 items/d[f]	≥ 8 items/d[f]	≤ 3 items/d[f]	≥ 8 items/d[f]

[a]HEI= Healthy Eating Index.
[b]Proportional scores were assigned to consumption levels between the minimum and maximum range.
[d]Maximum total HEI score is 100.
[e]Number of servings depend on recommended Food Guidelines for Turkish[9]
[f]Number of servings depend on recommended Food Guidelines for USA

consumed. Intermediate scores are calculated proportionally. The next four components measure compliance to recommended intakes of total fat, saturated fat, cholesterol, and sodium. The score of 10 on these components is reached by consuming at or below the recommended maximum levels. See Table 1. for the scoring criteria for each component.[10] HEI scores were analyzed continuously and were categorized into three groups (≤50, 51 to 80 and >80). A total score of more than 80 was considered "good", scores of 51-80 indicated "needs improvement", and scores of less than 50 were considered "poor".[11]

Anthropometric measurements

All anthropometric measurements were conducted according to World Health Organization standards and made in triplicate by nutritionists. The body mass index (BMI) was calculated from measurements of height and weight. Participants were classified according to BMI; those between 18.5 to 24.9 kg/m^2 were classified as normal weight, 25 or greater as overweight.[12] Fat mass and lean body mass were determined by bioelectrical impedance analysis with a TBF-300 Body Composition Analyzer (TANITA, Tokyo, Japan) according to the manufacturer's internal algorithm. Waist and hip circumferences were measured by trained personnel using a tape measure. Skin fold thickness (biceps, triceps, sub-scapular and supra-iliac) were measured three times on the right side of the body using a Harpenden caliper (Holtain, Crymych, UK).

Biochemical determinations

In order to evaluate the health conditions of adolescents related to nutrition, pre-prandial blood glucose, total cholesterol, triglyceride, albumen, BUN, ALT and AST, vitamin B_{12}, zinc, calcium, uric acid, total protein, iron and iron binding capacity were determined by the usual laboratories at the hospital.

Statistical analyses

All data analysis was performed by using SPSS statistical package (version 11.0) and the level of statistical significance for analysis was set at $p<0.05$.The descriptive statistics of continuous variables was expressed as mean ± standard deviation (SD). The differences between groups mean values were determined by parametric (Independent Sample t-Test) and non-parametric (Kruskal-Wallis) tests.

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Results

It has been determined that 72,1% of males participating in the research are in the 13-15 age group and 55,4% of females are in the 16-18 age group. 64.3% of males continue primary school, 33.1% to high school, and 2.6% to university. 53% of females continue high school, 45.8% to primary school and 1.2% to university. When the family backgrounds of the adolescents are examined, it has been determined that 28.8% of males' parents are separated, 19.1% have only a father, 17.4% have only a mother; and 31.3% of the females have only a mother, 26.6% of the females' parents are separated, 16.9% have only a father.

When the HEI scores are examined, 15.7% of males is (n=18) "bad", 80.9% (n=93) is "medium" and only 3.5% has a "good" score. On the other hand, the HEI score of 13.3% of the females (n=11) is "bad", 84.3% is (n=11) "low" and only 3.0% had a "good" score. The difference between the HEI scores of male and female adolescents has not been found to be significant ($p>0.05$). It was expected that the eating habits of these adolescents deprived of a family concept wouldn't be good either. Results of a research conducted on 16202 (8677 girls, 7525 boys) adolescents show that family dinner is associated with some healthful dietary patterns. Increasing frequency of family dinner was associated with higher consumption of fruits and vegetables and several beneficial nutrients, including fiber, folate, calcium, iron, and vitamins B_6, B_{12}, C, and E.[2] In a study[13], where the socio-demographic information and HEI scores of 1504 adolescents are compared, a decrease has been observed in the HEI scores according to the difference in the socio-demographic characteristics of adolescents accepted in the study. It is seen that, the study of Goodwin et al.[13] and the findings of this study support one another.

When the distribution of anthropometric measurements of adolescents according to percentiles is examined (Table 2), it has been found that the body weight of 61.2%, height of 53.0%, BMI of 62.1%, UCAC of 33.3%, triceps of 66.2% and sub scapular skinfold thickness measurements of 78.4% is normal. In the studies conducted on adolescents living in orphanages, it has been found that the rate of those whose body weight is at normal percentiles is 62.7-88.2%; the rate of those whose height is at normal percentiles is 59.7-77.0%; the rate of those whose BMI is at normal percentiles is 70.1%; the rate of those whose UCAC is at normal percentiles is 29.8-74.7%.[14-16]

In the studies conducted on adolescents living with their families, it has been found that the rate of those whose body weight is at normal percentiles is 70.8-72.4; the rate of those whose height is at normal percentiles is 64.4%; the rate of those whose BMI is at normal percentiles is 60-71.7%; the rate of those whose UCAC is at normal percentiles is 38.2%; the rate of those whose triceps skin fold thickness measurements are at normal percentiles is 75.1-77.8%.[17-20]

*Table 2. The Distribution of some Anthropometric Measurements of Adolescents according to Percentiles (NHANES)**

	Body Weight (percentile)									
	<5.		5-15.		16-85.		86-95.		>95.	
	No	%	No	%	No	%	No	%	No	%
Body weight	37	18.7	29	14.6	121	61.2	7	3.5	4	2.0
Height	60	30.3	28	14.1	105	53.0	2	1	3	1.5
BMI	20	10.1	40	20.2	123	62.1	7	3.5	8	4.0
UCAC[a]	95	48.0	30	15.2	66	33.3	6	3	1	0.5
Triceps ST[b]	28	14.1	25	12.6	131	66.2	11	5.6	3	1.5
Sub scapular ST[b]	9	4.5	20	10.1	155	78.4	9	4.5	5	2.5

** Line percentage has been taken.*
[a] Up center arm circumference
[b] Skinfold Thickness

Table 3. The Comparing of average biochemical findings of the adolescents participating in the research according to their gender (X±SD)

Biochemical Findings	Male (n=115)	Female (n=83)	Levene's test		t-test		Mann-Whitney U	
	X±Ss	X±Ss	F	p	T	p	MWU	P
Diastolic blood pressure (mmHg)	59.9±11.83	60.8±7.82	4.655	0.032	-0.658	0.511	4153.5	0.119
Systolic blood pressure (mmHg)	106.4±17.69	106.2±11.58	6.937	0.009	0.083	0.934	4589.5	0.645
Glucose (mg/dL)	92.1±10.33	85.7±9.86	1.449	0.230	4.400	0.000	-	-
BU (mg/dL)	15.7±3.21	13.2±3.43	0.050	0.824	5.160	0.000	-	-
ALT (U/L)	17.3±5.28	17.5±5.40	0.055	0.814	-0.235	0.814	-	-
AST (U/L)	25.0±7.24	25.3±7.42	0.064	0.800	-0.323	0.747	-	-
Calcium (mg/dL)	9.7±0.26	9.8±0.26	0.028	0.867	-2.392	0.018	-	-
Cholesterol (mg/dL)	166.8±26.88	170.7±27.91	0.011	0.918	-0.991	0.323	-	-
Triglyceride (mg/dL)	89.3±40.12	80.8±27.98	5.336	0.022	1.647	0.101	4326.0	0.261
Uric acid (mg/dL)	5.5±1.15	5.3±0.87	8.810	0.003	1.372	0.172	4226.5	0.170
Protein (g/dL)	7.9±0.75	7.5±0.66	3.579	0.060	3.685	0.000	-	-
Albumen (g/dL)	4.4±0.30	4.2±0.28	2.248	0.135	6.064	0.000	-	-
Zinc (ug/dL)	93.6±8.00	93.0±7.91	0,005	0.942	0.544	0.587	-	-
Iron (ug/dL)	81.6±23.50	81.3±31.46	10.95	0.001	0.059	0.953	4770.5	0.996
Iron binding capacity (ug/dL)	400.1±41.81	420.1±51.50	6.563	0.011	-3.009	0.003	3805.5	0.015
Vitamin B_{12} (pg/mL)	267.3±56.56	249.2±41.84	8.880	0.003	2.465	0.015	3982.0	0.047

Mann-Whitney U $p<0.05$ has been accepted important.

When the findings of this study are compared with other studies conducted on adolescents living in orphanages or with their families, it has been seen that it is at a lower level.

It has been observed that blood findings of the adolescents under the research are within reference values. It has been determined that difference between serum glucose, BUN, calcium, protein, albumen, iron binding capacity ($p<0.001$) and vitamin B_{12} ($p<0.005$) levels is significant (Table 3). In a research by Monge et al.[21] it has been reported that 31% of adolescents has a deficiency in vitamin B_{12}.

As it can be seen in Table 4, although a significant difference between HEI level and anthropometric measurements have not been determined statistically, those adolescents whose HEI levels are good have been found to be in a positive direction in terms of BMI, body fat percentage, body adipose tissue amount, body lean tissue amount, top center arm circumference, body weight, waist-hip circumference, triceps, suprailiac and sub scapular skin fold thickness when compared to those whose HEI levels are bad or medium. This situation can demonstrate that body components are affected in a positive way with the improvement in diet quality.

In a research[22] where**,** evaluated beverage samples, diet quality and characteristics of 2-11 year old children in terms of BMI, the beverage types and amount of school children in terms of BMI has demonstrated a significant difference. It has been reported that choice of beverages affect the diet quality and healthy eating of the pre-school and school children group; whereas, it has been also reported that the BMI of beverage amount and variety is effected only in school children group. In a study they conducted, Taveras et al.[23] have determined the BMI averages of those who never eat or who eat just fried meals once or less outside of the home is 19.1 kg/m^{2,} those who consume them 3 times a week is 19.2 kg/m^{2,} those who consume them 4-7 times a week is 19.3 kg/m^2. It has been reported in the research that consuming fried meals outside of the home actually causes the individual to intake sugar and excessive energy. It has been reported that excess energy intake at these ages can cause obesity in the future years.

Table 4. The average of the anthropometric measurements according to the Healthy Eating Index (HEI) of Adolescents

Anthropometric Measurements	HEI SCORE			Kruskal Wallis	
	Bad (n=29)	Medium (n=163)	Good (n=6)		
	X±Ss	X±Ss	X±Ss	Khi-square	p
BMI (kg/m^2)	19.9±2.59	19.9±3.28	22.0±9.42	0.277	0.871
Energy spent for physical activity (kcal)	1538.10±221.42	1477.26±253.74	1549.00±178.94	3.185	0.203
Fat (%)	6.0±3.29	7.4±5.4	11.1±12.0	1.249	0.535
Adipose tissue (kg)	11.7±5.5	13.8±7.5	16.7±13.9	0.866	0.649
Lean tissue (kg)	45.9±10.4	43.2±9.9	45.6±8.11	2.587	0.274
UCAC[a] (cm)	22.5±3.6	22.2±3.3	24.0±6.6	0.390	0.823
Height (cm)	160.0±125	158.2±11.9	158.8±9.9	0.791	0.673
Weight (kg)	52.9±11.8	51.1±12.6	56.6±16.5	1.662	0.436
Waist circumference (cm)	69.4±8.7	71.3±10.3	75.4±17.6	0.910	0.634
Hip circumference (cm)	83.2±8.9	83.6±10.6	89.1±15.5	0.706	0.703
Waist/Hip	0.83±0.03	0.85±0.06	0.83±0.04	3.876	0.144
Triceps ST[b] (cm)	9.5±5.27	10.7±5.95	17.7±16.0	2.647	0.266
Biceps ST[b] (cm)	4.87±1.90	5.70±3.04	8.28±6.63	2.442	0.295
Suprailiac ST[b] (cm)	13.4±7.3	15.03±10.4	22.2±20.1	0.109	0.947
Sub scapular (cm)	9.2±4.3	10.2±6.1	17.1±18.1	0.114	0.944

t-test $p< 0.05$ has been accepted to be important.

[a] Up center arm circumference

[b] Skinfold thickness

Although a significant difference has not been found statistically when the blood findings of adolescents in terms of HEI scores are examined, the serum calcium, B_{12} and triglyceride levels of the group whose HEI level is stated as good has been found to be low and cholesterol and iron binding capacity has been found to be high.

In the study conducted by Washi and Ageib[24], out of the adolescents whose diet quality and eating habits are bad, the blood glucose level of 80.8%, triglyceride level of 83.7%, hemoglobin level of 46.0%, blood calcium level of 68.6% and blood iron level of 64.9% have been found to be within normal limits. The biochemical data of our study and the above stated study support one another.

In a study conducted by Weinstein et al.[10] a positive correlation has been found in the HEI scores and serum, (r=0.25) and red blood cells (r=0.27), serum folic acid rate, serum vitamin C (r=0.30) and vitamin E (r=0.21) concentrations. In the same study, it has been determined that the most significant nutrition in the relation between healthy eating index score and nutritional element concentration are vegetables and fruit.

Discussion

In this study where the nutrition and health conditions of adolescents living in orphanages are examined, it has been determined that 27.8% of the adolescents' parents are separated. Only 3.0% of adolescents' HEI scores have been evaluated to be "good". The statistical difference between HEI conditions of male and female adolescents have not found to be significant (p>0.05). A large number of research have put forward that the Notion of family and a steady family life supports developing positive eating habits on adolescents.[1-3] It is believed that individuals lacking a family are unlikely to have good eating habits. Furthermore, studies suggest that dietary behaviors developed in adolescence are influenced by personal, family, and social factors and continue into adulthood, thereby increasing the risk for chronic disease later in life.[13,25]

As it can be seen in the study by Washi and Ageib[24] and in the biochemical data of this study, it has been determined that the biochemical findings of adolescents with bad diet quality and bad eating habits are within the reference values. Although these findings have been found to be within normal values, it is believed that these values are within limit reference values and that these values will bring a large number of health problems while moving on to adulthood.

Although a significant difference between the anthropometric measurements according to HEI levels has not been found, the BMI, body adipose percentage, body adipose tissue amount, body lean tissue amount, top center arm circumference, body weight, waist-hip circumference, triceps, suprailiac and sub scapular skin fold thicknesses are found to be higher compared to those whose HEI levels are bad or medium.

A significant difference between the average of the blood findings according to the HEI levels of adolescents have not been found (p>0.05).

As a result, it has been found that adolescents living in orphanages don't have healthy eating habits; that their blood signs are within the reference values and that the number of adolescents whose anthropometric measurements are within the normal limits is lower when compared with adolescents living with their families. In order to increase the HEI scores of adolescents living in orphanages, it has been suggested to re-plan the batch nutrition services to increase cereal, milk, meat, fruit and vegetable consumption within consumer satisfaction limits, to establish food served according to elective menus to ensure sufficient and balanced nutrition, to have the menus prepared by experts, to include adolescents whose body weight under 5 percentile to remedial-reformative applications and to have them followed by health medical personnel, to have health check-ups at certain intervals, to decrease illnesses and to conduct health support activities in order to improve their health conditions.

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The investigation of the proprioception in patients with Patello femoral pain: Using the sense of force accuracy

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Abstract

Background: The PatelloFemoral Pain Syndrome (PFPS) is the common musculoskeletal disorder and proprioception of knee can be changed in this group of patients. The sense of force is an important part of proprioception and fewer studies evaluated acuity of sense of force in patients with PFPS.

Aim: Comparison of the ability of reproducing isometric quadriceps force in patients with PFPS and healthy subjects.

Design: The Case – Control Study.

Setting: Patients with PFPS and control group.

Population: Seventeen patients with PFPS and seventeen matched healthy individuals.

Method: The Ipsilateral limb Matching (ILM) method was utilized to assess the acuity of the sense of isometric force in the quadriceps muscle. At first, participants produced 20 and 60% of quadriceps Maximal Voluntary isometric Contraction (MVC) using an isokinetic dynamometer and visual feedback and then reproduced the target forces without visual feedback. They were asked to estimate and reproduce the target forces based on their own perception of the quadriceps force during the isometric contraction. This test was performed in 20 and 60 degrees of knee flexion. The absolute error (AE), Constant error (CE) and Variable error have been used to measure the sense of force errors.

Results: The results of the three - way ANOVA conformed the significantly difference in AE (p=0.05 F value=8.29) and VE (p= .00 F value=55.50) in two groups. No significant was in CE in two group (P= .26 F value=1.25), but the patients group overestimated the target forces as compared to control group. The significant different in interaction knee position × group showed that the effect of knee position on sense of forces errors was not same in two groups and the patients with PFPS has more AE, CE and VE in 60 degree of knee flexion.

Discussion: The sense of force is possibly impaired in patients with PFPS because of Pain and Abnormal afferents form muscle receptors. More studied needed to evaluate of force reproduction ability to measure the knee proprioception.

Clinical Rehabilitation Impact: According to sense of force defect in patients with patellofemoral pain syndrome, the importance of muscle roll in knee proprioception should be considered in proprioception training programs.

Key words: Proprioception, sense of force, patellofemoral pain syndrome.

Introduction

Twenty five percent of knee complications are related to PatelloFemoral Pain Syndrome (PFPS), which is more prevalent in active adults, runners and young people, especially females (1-3). The most important clinical sign of this syndrome is pain in the anterior aspect of the knee, including patella and the surrounding retinaculum, during descending and ascending stairs, running and consistent cross-legged sitting (4-6). This syndrome is caused by imbalances in the forces controlling patella tracking during knee flexion and extension, particularly with overloading of the joint (7). Proprioception, which is an important factor in dynamic stability of the knee, is also a primary element in the motor control plans (8). Joint

injuries can modify the afferent information sent by the mechanical receptors, especially receptors of the Golgi tendon organ and muscle spindles, directly or indirectly (9) .In patients with PFPS, neuromuscular control and proprioception will sustain change due to pain and abnormal mechanical stresses on the soft tissue around the joint (9). Proprioception includes afferent information from internal peripheral areas of body and participate in postural control, joint stability and different conscious sensation (8). According to Sherrington, proprioception consists of three fundamental elements including: sense of position, kinesthesia and sense of force or tension (8, 10). In most of the recent studies, the Joint Position Sense (JPS) has been utilized to examine the proprioception precision of the knee joint, and the third element (sense of force) has been neglected (9, 11-14). Kramer et al found no significantly different in angle reproduction errors of the four knee flexion angles in healthy and PFPS individuals (14). In another study by Backer et al, proprioception complications were found in PFPS patients in several knee flexion angles (13). Sense of force is the conception of the within muscle force or tension during contraction, and is an important factor in the quality of motor function (15).

There are two possible mechanisms for estimating the amount of muscle force: (a) Central conception of the force, which happens on the basis of corollary discharge following sending the efferent copy from the motor cortex to the sensory cortex, (b) Peripheral mechanism, in which the force conception is earned upon the peripheral information sent by muscle mechanoreceptors, especially the Golgi tendon organs. Both of these mechanisms are involved in force conception (15-20). The combination of central and peripheral mechanisms used to correct the movement's errors during functional activates. During this process, the afferents are compared with the efferent copy and any mismatching between two information sources leads to changing the movement programs (21).

The quadriceps muscles play an important role in the activities of daily living like standing up and descending stairs. PFPS patients use this muscle less to avoid the pain and decreasing in quadriceps electromyographic (EMG) activity has been reported in some studies (22-24). This impairment may change the afferent signals from the muscle receptors and subsequently reduce the sensorymotor role of the muscle (25, 26). As muscle receptors have a main role in proprioception, the quadriceps impairment in PFPS patients could affect the proprioceptive acuity. It has been shown that PFPS patients cannot detect the damaging forces applied to the lower limb during walking; which may be due to decreased proprioceptive acuity (13). Therefore investigation the sense of force equity in patients with PPS may lead to a better understanding of its underlying mechanisms and possibly help clinicians with providing appropriate preventive protocols. Therefore, the purpose of the present study was to investigate the ability of reproducing the isometric submaximal force of quadriceps muscle in patients with PFPS.

Material and methods

Subjects

Seventeen females with PFPS and 17 asymptomatic matched controls were selected and signed an informed consent form. The protocol of the study was approved by the Ethics Committee of Tehran University of Medical Sciences. The control and patient groups were matched for height, age (19-39 years), weight, and lower limb dominancy. In patients with bilateral PFSP, the most painful side has been selected for test. The inclusion criteria for the patient group included: 1) history of anterior knee pain for 6 to 12 months; 2) positive Clarke's sign; and 3) anterior or medial knee pain during at least three of the following activities: ascending and descending stairs, continuous sitting with flexed knees, cross-legged sitting or squatting, and prolonged jogging or walking (14, 27). The intensity of anterior knee pain during the above-mentioned activities was measured using the Visual Analogue Scale (VAS) (28). The functional ability of patients was measured with the Kujula PatelloFemoral Disorder Scale Questionnaire that consists of 13 questions on daily activities. The score of questionnaire ranged from 0-100 and a higher score indicated more ability (29, 30). The healthy group had no history of anterior knee pain. Participants in both groups were excluded due to the following exclusion criteria: articular ligament and meniscus injuries, subluxation of the patella, cardiovascular problems, neuromuscular compli-

cations in lower limb, and a history of continuous sports activity in the past 6 months (2, 3, 13).

Sense of Force Testing

The assessment of the sense of force was done using the Biodex Isokinetic Dynamometer (Biodex Medical Systems, Shirley, New York, USA). During the trials, the knee joint was placed at either 20 or 60 degrees of knee flexion, because in most of daily activates the knee flexion angle is near 20 and 60 degree (1). In 20 and 60 % of MVIC (Maximum Voluntary Isometric Contraction) , as the minimal and submaximal quadriceps contraction, were selected to assess the sense of force (31). Therefore the subjects were participated in four trials (60º knee flexion 60 % MVIC, 60º knee flexion 20% MVIC, 20º knee flexion 60% MVIC, and 20 º knee flexion 20% MVIC) in random order. In order to maintain the length – tension relationship of quadriceps muscles, the hip joint was adjusted to 130º flexion (6). The resistance pad was placed on the intersection of 80 and 20 percent of the length of subjects s' lower leg. Then the subjects performed maximum static quadriceps contraction for two times with 180 seconds rest and the highest force amount was recorded as the (MVIC) (32). For any individual, the target force was calculated as the 20 or 60 % of the MVIC. In the present study, the Ipsilateral limb Matching (ILM) method was utilized to assess the sense of static force reproduction acuity in the quadriceps muscle; that is, in both groups, the subjects generated the selected muscle force (20 or 60 % of the MVIC) using visual feedback during 5 seconds. After 5 seconds interval, with closed eyes and upon their own perception of the muscle tension, the subjects estimated and reproduced the same amount of force (16, 33-36) . The subjects have expressed when they reproduced the target forces. Each trial has been repeated 3 times with 5 minutes interval to decrease the force matching errors (36). Participants were instructed to keep their mind on the amount of tension as well as the within muscle force of the quadriceps while reproducing the target force. Moreover, each subject was given two orientation trials for force reproduction before the test (19, 35). Subjects rested for 3 to 5 minutes between each trial to avoid fatigue (36). The dynamometer presented the estimated force generated by the subjects through a torque-time curve. The important point was that the participants did not have any pain or discomfort during the test.

To measure inter – session reliability, 4 patients and 4 healthy controls were recruited. The sense of force test was repeated after an interval of 2 days.

Computation of the Sense of Force Errors

The Software developed by the Biomechanics engineering, used to analysis the reproduced forces. This designed with MATLAB and presented the exported files of the dynamometer as a torque-time graph. Moreover, it could provide averages out of the portions of the graph along which the subject acquired and maintained a constant level of target force (Figure 1). The average estimated force of any subject was computed for 20 and 60 degrees of knee flexion. To assess the acuity of the sense of force, Constant Error (CE), Absolute Error (AE), and Variability Error (VE) were used (37). The definition of mentioned errors is described below:

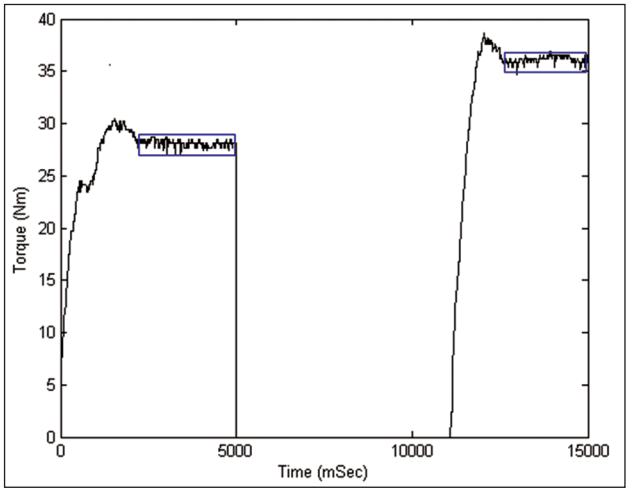

Figure 1. Time – Torque curve of quadriceps isometric force during sense of force test. The oblong box is the part of curve that the subjects maintained the target force in constant level. The software measured the average of torque in oblong box

AE: measures the overall accuracy in performance. It is the average absolute deviation between the estimated force and the target force without considering overestimation or underestimation of the force.

$$AE = \frac{|xi - T|}{n}$$ (Xi=estimated force, T= target force, n= number of estimation)

CE: simple average between the estimated force and the target force with considering the overestimation and underestimation of the force.

$$CE = \frac{xi - T}{n}$$ (Xi=estimated force, T= target force, n= number of estimation)

VE measures the consistency in estimated forces during three repetitions.

$$VE = \sqrt{\sum (xi - M)^2 / n}$$ Xi=estimated force, M= mean of 3 estimated forces, n= number of estimation)

Statistical analysis

Statistical Package for the Social Sciences (SPSS) for windows (version 16.0, SPSS Inc., Chicago, IL, United States) was used for statistical analysis. Prior to analysis, the data were tested for normal distribution using the Kolmogorov–Smirnov test with the significance level set at 0.05. Three- way ANOVA was used to compare the independent variable (group, knee flexion angle, target force level) in four trial of sense of force test and investigate the effects of knee position and level of force target on amount of force reproduction errors. The ICC (interclass correlation coefficient) was computed for force reproduction errors.

Results

There was no significant difference between two groups in age, weight and height. Table 1 shows the demographic data, VAS and functional ability scores of subjects in two groups.

According to table 2, the results of three - way ANOVA showed significant difference between two groups in AE. As seen in figure 2, there was significant difference in Knee position × Group interaction and force level × group interaction. It means that the effects of knee position and force level on AE were not same in two groups. However, the patients with PFPS had more AE in 60 degree of knee flexion. The healthy subjects had more AE in 20 degree of knee flexion.

Table 1. Descriptive statistical in healthy and patients groups

Variables	Patients Group (n :17)	Healthy group (n: 17)
Age	22.35 ± 1.9	22 .10 ± 1.84
weight	57.06 ± 5.75	56.00 ± 6.16
height	162.29 ± 4.51	162.04 ± 5.05
CVA	4.37 ± 1.69	0.00
Kujula Questioner Score	82.16 ± 5.27	105.00

Table 2. Three – way analysis results for effects of knee position and level of target force on Absolute Error (AE)

Source of Errors	P Value	F value
Knee position	0.02	5.59
Force level	0.00	34.40
Group	0.003	8.86
Knee position and force level interaction	0.68	0.16
Knee position and groups interaction	0.03	4.43
Force level and group interaction	0.03	4.34
Knee position and force level and group interaction	0.05	3.57

Table 3. Three – way analysis results for effects of knee position and level of target force on Constant Error (CE)

Source of errors	P Value	F value
Knee position	0.76	0.08
Level of target force	0.49	0.47
Group	0.26	1.25
Knee position and force level interaction	0.36	0.82
Knee position and groups interaction	0.02	5.39
Force level and group interaction	0.34	0.62
Knee position and force level and group interaction	0.06	4.64

There was no significant difference between two groups in CE (table 3). There was significant difference in knee position × group interaction and it showed that, the patient with PFPS has more CE in 60 degree of knee flexion (figure 3). The patients group overestimated the target force as compare to healthy subjects.

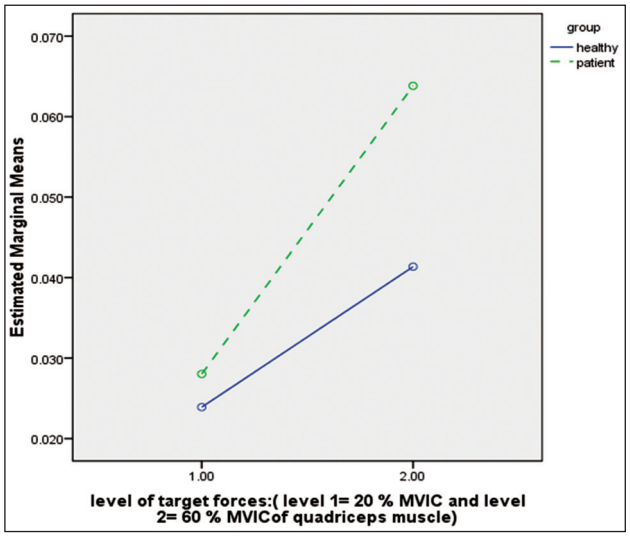

Figure 2. The Force level and Group interaction Absolute Error (AE). The patients group had more error in 60 degree of knee flexion

As depicted in table 4, there was significant difference in two groups in VE. The knee position × group interaction was significantly different and the patients with PFPS presented the more VE in 60 degree of knee flexion (figure 4). According to results of force level × group and knee position × Group interactions, changing the knee joint angle has affected the sense of force errors more than changing the level of target force.

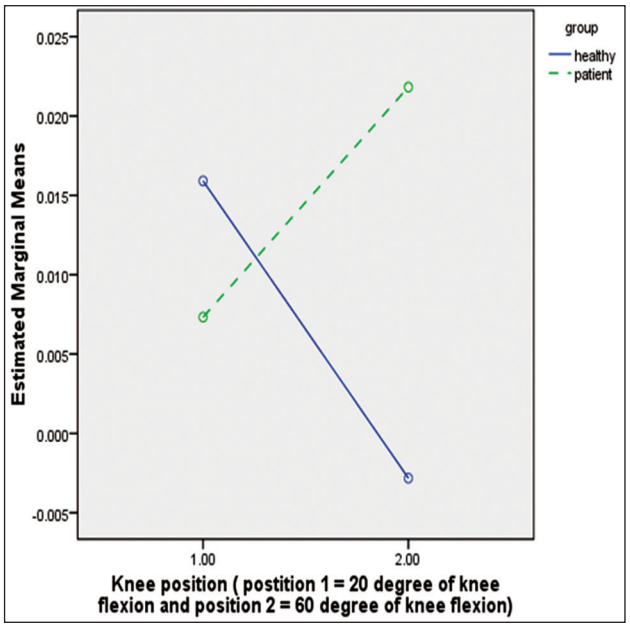

Figure 3. The Knee position and Group interaction on Constant Error (CE). The patients group had more error in 60 degree of knee flexion and they overestimated the target force

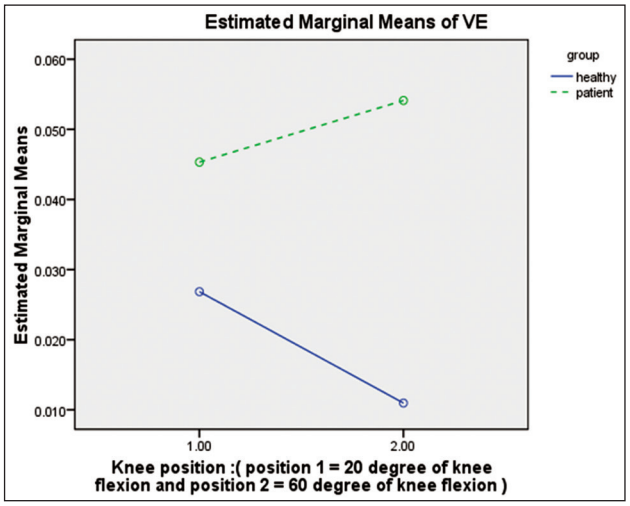

Figure 4. The knee position and Group interaction Variable Error (VE). The patients group had more error in 60 degree of knee flexion

Table 4. Three – way analysis results for effects of knee position and level of target force on Variable Error (VE)

Source	P Value	F value
Knee position	0.39	0.37
Level of target force	0.04	4.29
Patient and healthy group	0.00	55.50
Knee position and force level interaction	0.63	0.22
Knee position and groups interaction	0.00	8.90
Force level and group interaction	0.19	1.70
Knee position and force level and group interaction	0.73	0.11

Discussion

The aim of this study was to compare the sense of force acuity between patients with PFPS and healthy controls. According to the findings of the present study, the sense of force, one of the important aspects of proprioception, is possibly impaired in patients with PFPS, similar to JPS and kinesthesia which has been shown to be disturbed in studies conducted by Kramar et al (14), Backer et al (13) and Akseki et al (11). The results of present study showed that patients with PFPS had more error in reproducing the target force in the middle knee range of motion. This finding is consistent with the findings of Backer who reported that the patients with PFPS had errors in the joint position sense in 60 degrees knee flexion under non-weight bearing conditions (13).Probably, Pain in the midrange of knee flexion in daily activities may gradually result in decreased proprioception and the sense of force (38, 39). In addition, the roll of muscle spindles and the Golgi tendon organ in knee proprioception is dominant in the midrange of knee flexion while in the end ranges of knee movements; the non-contraction receptors play an important role in knee proprioception. Changes in proprioception following pain or impairment may increase proprioception test errors (40). Also, during knee flexion, abnormal tissue stresses arising from mal tracking of the patella may lead to abnormal proprioception in patients with PFPS (13).

Quadriceps has a significant part in the activities of daily living, particularly in controlling the sudden forces applied to the lower limb. Lower EMG activity of quadriceps muscles has been reported in patients with PFPS, as a pain relieving mechanism that affects the quadriceps mechanoreceptors and possibly modifies the afferent input to the higher centers (23, 41). The less accurate sense of force in PFPS participants could be justified by this hypothesis. Generally, quadriceps neuromuscular control complications can start with abnormal proprioceptive feedbacks from mechanoreceptors around the patellofemoral joint and become worst afterwards. Moreover, sending such information encourages changes of sensory information and abnormal discharge in the thick fibers of proprioceptive receptors (13, 16)

The finding of the present study suggested that patients with PFPS overestimated the target force during the reproduction of the force. Nowak studied the hand finger force control ability of patients with sensory neuropathy and suggested that lack of peripheral sensory information would lead to more attention to central force perception signals and as a result, the patients would overestimate the target (42). It seems that the relationship between central and peripheral mechanisms of the sense of force is disturbed in this group. This evidence comes from observations of force estimation errors in pathological conditions or fatigued muscles (17).

The most important finding in this study was significantly difference in VE in two groups. It means that the patients with PFPS have produced heterogeneous force during three estimation trials. Consistency and continuity in generating static force in several repetitions is necessary in many of the motor skills and the extent of variability error measures this skill (26). Rubley et al studied the effect of cold therapy on the sense of force in the thumb finger and found decreased afferents of muscle and skin proprioception receptors would increase the variability error during the force reproduction test (26). The exact source of variability error during force reproduction trial is unknown but it depended upon muscle condition, irritability of motor neurons or changes in the efferent nerve fibers from the central nerve system (26). Probably, the proprioception afferents of GTO and muscle receptors are necessary to tuning the submaximal muscle force during different activities and the high variable error in force estimation of patients with sensory neuropathy confirms this hypothesis (25). In present study, the healthy subjects had more AE in initial knee flexion (20 degree of knee flexion). Cafarelli investigated the roll of joint position on muscle force production capacity and sense of force and reported that changes of the joint angle causes changes in the length-tension relation in the muscles which alters the force generating capacity and sense of force precision (21). Probably, changing the length – tension relationship in 20 degree of knee flexion led to decrease the sense of force accuracy (21). One of the important sources of errors during force reproduction trial is training the subjects to pay attention to muscle force while estimating the muscle force. Due to the psychophysiological nature of the "sense of force", it is possible that despite training the subjects, both peripheral and central mechanisms of the sense of force be shared during the force re-

production (36) ; however, the fact that we did not evaluate this hypothesis can be regarded as one of the limitations of this study.

Finally, there are two mechanisms for the detection of the sense of force: peripheral and central. The brain compares peripheral sensory signals with corollary discharges of the motor signals in the central nervous system in order to conceive the internal muscle force. In a normal muscle contraction, there is sufficient conformity between the two mentioned information sources, and any inconsistency among the peripheral biofeedback and copies of motor signals leads to the sense of a different amount of force and higher error in an individual (19).

Conclusions

According to Sense of force tests, the proprioception is disturbed in patients with PFPS. The evaluation of the ability of muscle force reproduction, along with other proprioception tests, can be used to measure patellofemoral joint proprioception but it needs to clarify the accurate methodology to measure the sense of force accuracy in knee joint.

Limitations

Our sample was exclusively composed of female subjects to avoid possible differences caused by gender. The lower number of participants was another limitation of this study. Using the surface electromyography during sense of force testing could determine the effects of muscle fatigue and co – contraction of muscle around knee joint on sense of force equity.. More studies are needed to measure the acuity of the sense of force in different pathologies of the knee and quadriceps muscle. In view of the importance of quadriceps muscle training, further studies are required to assess the sense of force in patients with PFPS.

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Levels of oxidative status and cancer markers in malignant Mesothelioma

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Abstract

Background: Malignant mesothelioma (MM) is a tumor arising from the serous membranes. Aim: The purpose of this study is to investigate the levels of cancer antigens, oxidative markers and several biomarkers in MM patients and controls.

Materials and methods: A total of 48 MM patients and 52 healthy control subjects were studied. In this study was measured the levels of total oxidant status (TOS), total antioxidant capacity (TAS), ceruloplasmin, transferrin, folic acid, ferritin, vitamin B12, carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 125, CA 15-3, CA 19-9 and cytokeratin fragment 19 (CYFRA).

Results: The mean age of MM patients was 55.8 ± 13.1 years and 81.3% of them had asbestos exposure. Among the MM patients, 91.7% had the pleural type and 68.8% were found the epithelial type.

TOS levels were found to be significantly higher in the MM than controls. However, CEA, folic acid, transferrin and ceruloplasmin serum levels were significantly higher in the control group than among the MM patients. Vitamin B12, CA 125, CYFRA and ferritin levels were significantly higher in the MM group than in the controls. The study found no difference between these laboratory findings and the stage, Karnofsky performance scores.

Conclusions: Cancer markers were elevated in the MM patients, but their CEA levels were reduced. A significant drop in a patient's CEA level may provide useful criteria for MM exclusion. In the MM group, oxidative stress was increased. New biomarkers may be useful for diagnosing MM in patients who had asbestos exposure and other risk factors.

Key words: Mesothelioma, markers, oxidative stress, TAS, TOS.

Introductıon

Malignant mesothelioma (MM) is a tumor that arises from the serous membranes of the pleura and, less frequently, of the peritoneal and pericardial cavities and the tunica vaginalis testis. Prognosis is poor, with median survival for the pleural form only about nine months following diagnosis.[1] The etiologic association with exposure to asbestos is well documented, including dose response parameters.[1,2]

Biomarkers have limited use for early detection of mesothelioma, among high-risk populations. Several markers, such as hyaluronic acid, tissue polypeptide antigen and ferritin, were found to distinguish mesothelioma from both the control subjects and those who had the benign form of the disease.[3–5]

A combination of cytokeratin fragment 19 (CYFRA 21-1) and tissue polypeptide antigen correlated with the estimated of prognosis in 52 patients.[6] Carbohydrate antigen 125 (CA-125) may be useful, but few MM studies have looked at this.[7] An ideal biomarker for MM has not been determined.

In general, elevated serum and/or pleural fluid carcinoembryonic antigen (CEA) excluded MM and such cases should be considered to be lung cancer.[8,9] In the study performed in Turkey showed that CEA levels in serum and pleural fluids and CA 19-9 levels in pleural fluids were lower in patients with malignant pleural mesothelioma (MPM) than among those with bronchial cancer. In addition, CA 15–3 levels in the pleural fluids of MPM patients were higher in patients with bronchial cancer.[10]

It is important to note that pleural mesothelioma patients who received folic acid and vitamin B12 supplements were better able to tolerate treatment. These patients had a median survival rate that was five months longer than patients who did not receive the supplements.[11]

Ferritin levels were found to be higher in malign pleural effusions than in benign pleural effu-

sions.[12] Also of significance is that the MM patients who were studied lived in a region where the use of environmental asbestos is common.[13-15]

The purpose of our study, then, is to investigate the levels of cancer antigens, oxidative markers and several biomarkers in MM patients and control subjects, by looking at MM patients living in a region where the use of environmental asbestos is common.

Materials and methods

The study included 48 MM patients and 52 healthy control subjects. Local ethics committee approval was obtained in this prospective study. All of the MM patients had proven histopathology, from the Dicle University Medical School Hospital. The healthy controls had no chronic diseases (e.g. chronic obstructive pulmonary disease, lung cancer, etc.).

Data for the mesothelioma patients were recorded on standard forms. Details about the patients' symptoms, asbestos exposure, methods of diagnosis and demographic and histopathological information were taken from their files.

Histological confirmation was investigated in the material, and only MM patients who had been histological proven were included the study. Histochemical or immunohistochemical stains were used if necessary, and clinical and radiographic variables were defined and measured at the time of diagnosis. Because some patients did not permit toracoscopy, MM staging was done according to the Butchart staging system.[16] Procedures for diagnosis were divided into two groups: non-invasive procedures (blindly needle biopsy and radiologic guided needle biopsy and invasive procedures (video associated toracoscopy and toracotomy).

Each blood sample that was collected was immediately centrifuged at 4000 rpm +4 C for 10 minutes and then transferred into an Eppendorf tube. Samples were transferred on ice and kept in –70 C deep-freeze until the end of the study, which was completed within three months. Total oxidant status (TOS) was measured by Erel's methods[17] and total antioxidant capacity (TAS) was evaluated through a novel automated and colorimetric measurement method developed by Erel.[18] Ceruloplasmin and transferrin levels were measured by Image 800 nephelometry (Beckman-Coulter; Fullerton, CA, USA). The chemiluniscence method, with Cobas (Roche Diagnostics GmbH, Mannheim, Germany), was used to measure CEA, CA 125, CA 15-3, CA 19-9, CYFRA, ferritin, folate and vitamin B12 levels.

Statistical Analysis: Parameters were recorded in the SPSS program. Mean (SD), median (interquartile range, IQR) and frequencies (%) were used to describe the characteristics of the patients. To compare categorical variables or continuous variables between the groups, the study used the X2 test or Fisher's exact test and the independent t test, Mann-Whitney-U test or Kruskal-Wallis test, respectively. $P < 0.05$ was taken for significance.

Results

The study included 48 MM patients and 52 healthy control subjects. The mean age of the MM patients was 55.8 ± 13.1 (24-79) years. A total of 19 of the MM patients (39.6%) were over 60 years of age. Among these patients, 24 were male and 24 female.

A total of 39 MM patients (81.3%) had environmental asbestos exposure. The mean environmental asbestos exposure time was 34.0 ± 15.9 years, and 30 (62.5%) patients had over 20 years of environmental asbestosis exposure.

Demographic data are presented in Table 1.

Table 1. Demographic data MM patients

Parameter	n	%
Mean age (years)	55.8 ± 13.1	
Asbestos exposure time (years)	34.0 ± 15.9	
Symptoms		
Dyspnea	42	87.5
Chest Pain	34	70.8
Weight Loss	7	14.5
Smoking		
No	29	60.4
≤ 20 years	7	14.5
>21 years	12	25.1
Stage		
Stage 1-2	38	79.2
Stage 3-4	10	20.8
Karnofsky performance score		
> 60	43	89.6
< 60	5	10.4

Of the MM patients, 44 (91.7%) had the pleural type of the disease. Two patients had peritoneal MM, one had pericardial MM and one had testicular MM. Non-invasive procedures were used for diagnosing 31 MM patients (64.6%) and invasive procedures for the other 17. Right side involvement was found in 26 patients and left side involvement in 18. The epithelial type of MM was found in 33 patients (68.8%). Cytology was positive in 13 of the MM patients (27.1%).

The mean symptom time before diagnosis was 5.3 ± 3.8 months. Dyspnea was the most frequently reported symptom (42 patients; 87.5%) (Table 1). The most frequent stages seen were stages 1 and 2.

TAS was found to be higher in the MM group than among the controls, but this difference was not significant (p=0.074). CA 15-3 and CA 19-9 levels were higher in the MM group than in the controls, but this difference was not significant either (p>0.05).

Table 2. Laboratory parameter of MM patients

Parameter	Group	n	Mean	±SD	p
TAS	MM	48	0.85	0.34	0.074
	Control	52	0.73	0.34	
TOS	MM	48	196.34	221.12	0.011
	Control	52	105.89	92.49	
Transferrin	MM	48	171.20	44.52	0.000
	Control	52	216.85	62.30	
B12	MM	48	441.69	147.22	0.000
	Control	52	195.00	72.12	
CA 125	MM	48	125.98	170.79	0.000
	Control	52	11.41	7.23	
CA 15-3	MM	48	56.22	58.54	0.2
	Control	52	45.12	12.09	
CA 19-9	MM	48	79.63	179.63	0.9
	Control	52	77.19	55.50	
CIFRA	MM	48	10.99	16.22	0.000
	Control	52	1.34	0.64	
Ferritin	MM	48	370.73	267.81	0.000
	Control	52	112.27	43.71	
Folate	MM	48	7.050	5.05	0.001
	Control	52	12.25	9.82	
CEA	MM	48	3.14	4.48	0.000
	Control	52	8.0	8.69	
CIFRA/CEA	MM	48	5.89	9.39	0.000
	Control	42	0.59	1.02	
CA 125/CEA	MM	48	89.69	105.14	0.000
	Control	42	4.46	6.57	

TOS was also found to be higher in the MM group than among the controls and this difference was significant (p=0.011). CEA, folic acid, transferrin and ceruloplasmin serum levels were significantly higher in the control group than among the MM patients (p<0.05).

The study found vitamin B12, CA 125, CYFRA and ferritin levels to be significantly higher in the MM group than among the controls (p=0.000). CA 125/CEA and CYFRA/CEA ratios were also significantly higher in the MM group than in the control group (P = 0.000). The CA 125/CEA ratio was substantially higher in the MM patients than among the controls, by almost 25 folders (p=0.000). These laboratory findings are presented in Table 2. The study found no difference between the laboratory findings and the stage, Karnofsky performance scores.

Discussion

In areas that contain a lot of asbestos, exposure to environmental asbestos and erionite starts at birth. Because asbestos exposure in the region studied is mainly environmental,[13–15] it begins at birth. Therefore, mesothelioma can be detected at earlier ages than in other areas. One study performed in this region found that the mean age of patients was 52.4 years.[13] The mean age for environmental asbestos cases in our study is similar.

One study found the level of CA 125 to be higher in MM patients, with mean levels of 18.8 ± 1.2 U/ml.[19] CA 125 is a well-defined cancer marker.[20] In other studies, CA 125 was at high levels, especially in patients who had high tumor burden.[21-23] The mean level of CA 125 in our study was found to be at very high levels in the MM patients, with 10 folder increases over the control group. The prognostic role of CA 125 in MM patients should be investigated. CA 125 levels in lung cancer patients were also found to be high, but these levels increased in some patients whose tumors progressed after the treatment.[24] Further investigation of this association may be interesting in terms of MM patients.

Levels of CEA, another cancer marker, generally showed decreased serum and/or pleural fluids in mesothelioma patients. Elevated CEA levels in serum and/or pleural fluids probably do not indicate MM and lung cancer should be considered.[8,9] In our study, CEA levels were low among the MM

patients. Similarly, CEA levels in serum and pleural fluids were found to be lower in patients with malignant pleural mesothelioma (MPM) than in those who had lung cancer and in other cancer patients[10,25] CA 19-9, which is also a cancer marker, was found to be raised in malign pleural effusion.[12]

In another study conducted in Turkey, CA 19-9 levels in pleural fluids were found to be lower in MPM patients than among bronchial cancer patients. In addition, CA 15-3 levels in pleural fluids among MPM patients were higher than in patients with bronchial cancer.[10] Our study found high levels of CA 19-9 and CA 15-3 in MM patients, but this difference was not significant. We believe that the role of these cancer markers in MM is complicated.

A combination of CYFRA and tissue polypeptide antigen correlated with prognosis in one study involving 52 patients, and this research also found that in multivariate analysis CYFRA has prognostic significance in MM patients [6]. In another study, CYFRA levels were found to be higher in MM patients than among the control subjects.[26] Our study found higher CYFRA levels in the MM group, with 10 folder increases. Although an ideal biomarker for MM has not been determined, CYFRA levels may be able to play a prognostic role in MM patients. Also, one study found higher CYFRA levels in mesothelioma patients than among patients with other cancers and, interestingly, CYFRA/CEA was also found to be higher in mesothelioma patients.[25] Our study found CYFRA/CEA and CA 125/CEA ratios to be significantly higher in the MM group. These ratios may be useful for a differential diagnosis of mesothelioma.

One study found ferritin levels to be higher in malign pleural effusions than in benign pleural effusions.[12] In another study, ferritin levels were higher in non-small cell and small cell lung cancer than among the controls, and ferritin levels were found to be associated with performance status. The authors believe that ferritin may be used as a prognostic factor in lung cancer.[27] Our study found higher levels of ferritin in the MM group than among the controls.

In one study, TAS levels were higher in cancer patients than among the controls.[28] Another study of COPD exacerbation found that the patients' TOS levels increased, and were higher at discharge than at admission as well as being higher than those of the control group. In addition, TAS was significantly lower at both time points in patients with COPD than among those in the control group. We were unable to find literature about TAS and TOS in MM patients. In our study, TOS levels were significantly higher in the MM patients, but TAS levels were not significant.

We believe that the oxidant/antioxidant imbalance might be significantly pronounced in patients with MM. Increased oxidative stress, elevated systemic inflammation and decreased antioxidant defense may play a role in MM inflammation. Detailed studies need to address this specific issue.

Pleural mesothelioma patients who received folic acid and vitamin B12 supplements were better able to tolerate treatment (low toxicity and more cycles of treatment). These patients had a median survival that was five months longer than patients who did not receive the supplements. The authors suggest that patients with pleural mesothelioma could benefit from single-agent pemetrexed treatment combined with vitamin supplementation (folic acid and vitamin B12).[11] Our study found that folic acid levels were significantly lower in the MM group than among the controls, but that vitamin B12 levels were lower in the control group. We believe that folic acid supplementation is needed for MM patients. However, supplementation with vitamin B12 is complicated, and large series studies are required.

Levels of acute-phase proteins, such as albumin and transferrin, decrease in response to any injury.[29] Yildirim et al. found serum transferrin levels to be lower both in non-small cell and small cell lung cancer patients than among controls.[27]

This study, however, has some limitations. First, the parameters analyzed diagnosis time and no follow-up was done. Therefore, we cannot study the association between these markers and MM prognosis. Also, subgroups cannot be analyzed because of the small number of them (stage, performance status, asbestos exposure, etc.).

Cancer markers are elevated in MM patients, but CEA levels are reduced. A significant drop in a patients' CEA level may provide useful criteria for MM exclusion. Also, because oxidative stress is increased in MM patients, its role should be investigated. An ideal biomarker for MM has not yet been determined. New biomarkers may be useful for a non-invasive diagnosis of MM.

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The comparison of graft alternatives in hand located enchondroma treatment

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Abstract

Purpose: The cavity formed after curettage is usually filled with autograft or allograft in hand located enchondromas. Demineralized bone matrix use is a novel treatment option as allograft. In this study, treatment options of demineralized bone graft and autograft were compared.

Method: A total of 31 patients (12 male, 19 female, mean age 25 years, range 11-58) who underwent operation due to hand located enchondroma between 1998-2011 were retrospectively evaluated. Autograft harvested from iliac crest was used in 17 patients in order to fill curettage cavity and demineralized bone matrix (DBM) was used in 14 patients as allograft. Histologic diagnosis of all cases was confirmed. Radiographic findings were evaluated according to Tordai classification and functional outcomes were evaluated according to scoring system of Enneking . Follow up period was meanly 6.5 years (range 2 months- 14 years) in autograft group and 1 year (range 2 months-2 years) in allograft group.

Results: Hand grip strength was regained in an average of 52 days and consolidation time was 42 days in autograft group. In allograft group, these durations were 52 and 43 days, respectively. Functional outcomes were found very good, excellent in 9 patients (53%) and good in 8 patients (47%) in autograft group. On radiographic assessment, 11 patients (64.7%) were in group I and 6 patients (35.3%9 were in group II according to Tordai classification. In allograft group, functional results were excellent, very good in 7 patients (50%) and good in 5 patients (35.7%) and poor in 2 patients (14.3%). According to Tordai classification, 8 patients (57.1%) were in group I , 4 patients (28.6%) in group II and 2 patients (14.3%) were in group III. Fracture was seen in only one case (5.8%) as recurrence and complication in autograft group.

Conclusions: A statistically and clinically significant difference was not found between the success of DBM applications as aoutograft or allograft in treatment of hand located enchondromas.

Key words: Hand enchondroma, curettage, autograft, allograft.

Introduction

Enchondroma is the most common primary bone tumor of the hand and the wrist and consists 40% of all enchondromas. They are most common in proximal phalanx, metacarp and medial phalanx, respectively (1,2). Diagnosis is usually made with admission due to painful or painless swelling, pathologic fracture or incidentally on radiographies (3-5). Radiographic appearance of the lesion shows central, well demarcated, radiolucency. Point calcification may be seen. Medullar radiance and cortical thinning is frequent (1,2). Enchondroma is immature hamartomatous accumulation of hyaline cartilage in the bone (6). The origin of this accumulation is the lacking of normal enchondral ossification under epiphyseal plates (7). Rare cases like distal phalanx, carpal bone location, pathologic fracture of navicular bone, traumatic deep flexor tendon avulsion development were also reported in literature (3-5).

The most common treatment method for enchondromas is surgical therapy. The surgical procedure is filling the cavity formed due to curettage using autograft, allograft or various osteoconductive materials. The aim of the therapy is to prevent fractures and deformities and to confirm the diagnosis histologically (8-12). A less commonly used treatment method is making only open or endoscopic curettage for the tumor in a certain location (13-17). Literature data is available about spongious bone use as allograft in surgical treatment and sufficient data is not available about clinical and radiologic outcomes of DBM use.

The aim of this study is to evaluate early and late clinical and radiologic outcomes of the group that DBM was used as allograft and autograft group.

Patients and method

A total of 31 patients (12 male, 19 female, mean age 25 years, range 11-58) who underwent operations due to enchondroma between February 1998 and July 2011 were evaluated retrospectively. Reasons for admission were deformity and pain in 12 patients (38.7%), only pain in 8 patients (25.8%), deformity and swelling in 5 patients (16.1%), pathologic fracture in 2 patients (6.5%) and 4 patients (12.9%) had no complaints.

Enchondromas were seen on the right in 64.5% of the patients, on the left in 33.5% and in the dominant hand in 48.4%. Hand located enchondromas were classified in 5 groups according to radiographic appearance by Takigawa (18).

Graft selection was done according to the effectiveness of the allograft, the amount of graft needed and age. Patients were divided to two groups according to graft options used after curettage. While autograft harvested from iliac crest was used for filling the cavity in 17 patients (figure 1 and 2), DBM was used as allograft in 14 patients(figure-3 and -4). While all patients in whom autograft was used were performed general anesthesia, local anesthesia was used in the patients in whom DBM was used. Digital anesthesia was used in 7 patients (50%), RIVA was used in 5 patients (35.7%) and axillary anesthesia was used in 2 patients (14.2%). Operations of only two patients were performed after complete healing of the fracture in both groups.

Age, gender, duration of hospital stay and follow up period in the patients in whom allograft or autograft was used are given in Table 2.

Table 1. Classification of lesions according to localizations (Takigawa classification)

	Autograft	Allograft
Central	11	8
Excentric	5	4
Associated	-	-
Multi-center	1	-
Giant	-	2

About four weeks of immobilization was applied by finger splint for medial and distal phalanx located ones and volar plaster splint for the others. Materials removed intraoperatively were examined histologically in all patients and diagnosis was verified. Postoperative complications and detected findings were determined. On follow up, findings on final radiographies were evlauted according to Tordai classification (14) and functional outcomes were evaluated according to functional scoring system of Enneking defined for each joint and approved by ISOLS (International Symposium on Limb Salvage) (19).

Approval was obtained from Local Clinical Researches and Ethics Committee.

Results

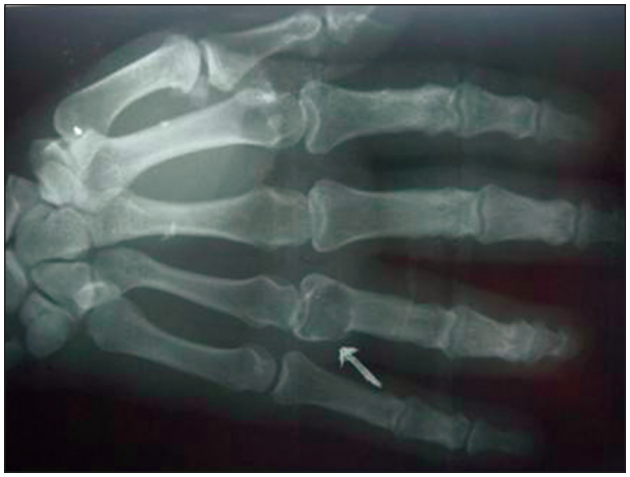
While the most common localization of enchondroma is proximal phalanx in the patients in whom autograft was used (11 patients, 64.72%), the most common localization was metacarp in the allograft group (6 patients, 43%).

Graft was seen to be consolidated in an average 42 days in autograft group. The time for full hand grip strength was mean 52 days. Pain in iliac crest lasting for 2 weeks was seen in 3 patients. Recurrence of enchondroma together with insufficient union in rotation, pain and refracture was seen in only one patient. Functional outcomes were found very good, excellent in 9 patients (53%) and good in 8 patients (47%). On radiographic assessment, 11 patients (64.7%) were in group I, 6 patients were in group II (35.3%) according to Tordai classification. Only one patient in autograft group admitted with limitation of movement in the third finger and numbness in the second and third fingers lasting for 6 months. No pathologies were found on EMG. Complaints decreased following medical treatment. In the DBM group was used as allograft, graft was seen to be consolidated on radiographies. Mean duration for full hand grip strength was 52 days. Functional outcomes were excellent, very good in 7 patients (50%), good in 5 patients (35.7%) and poor in 2 patients (14.3%). According to Tordai classification, 8 patients (57.1%) were in group I, 4 patients (28.6%) were in group II and 2 patients (14.3%) were in group III. A significant difference was not detected between groups in terms of hand grip strength and consolidation times and no statistically significant difference was observed. Although there was a only statistical difference between the autogreft used pa-

Table 2. Distribution of gender, age, duration of hospital stay and follow up between groups

	Group 1(n:17)	Group 2 (n:14)	P value
Age (year)	27,6 ± 9,5	23,5±12,0	0,29
Gender (male)	7(%41)	9(%64)	0,18
Duration of hospital stay (day)	4,8± 1,7	5±3,0	0,84
Follow up	76 ± 44,8	10,4 ± 6,0	<0,001

tients with right hand mass and DBM used with left hand mass, there was not any clinical difference.



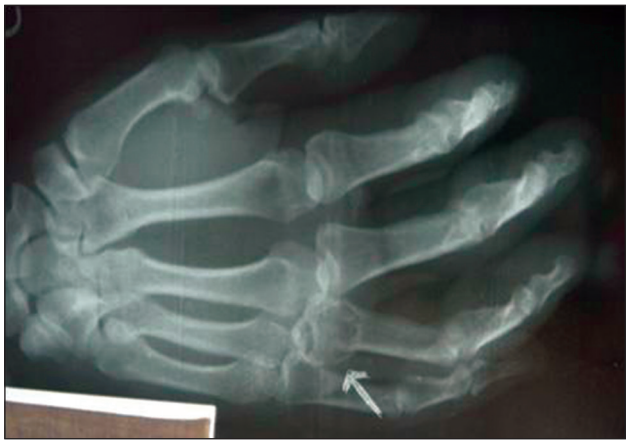
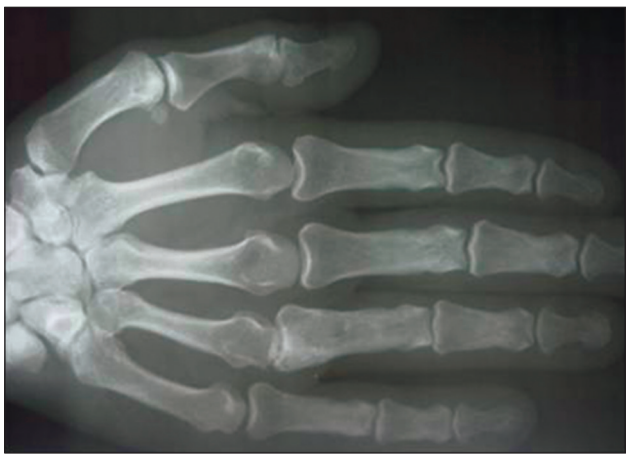


Figure 1. Preoperative AP and Oblique graphy



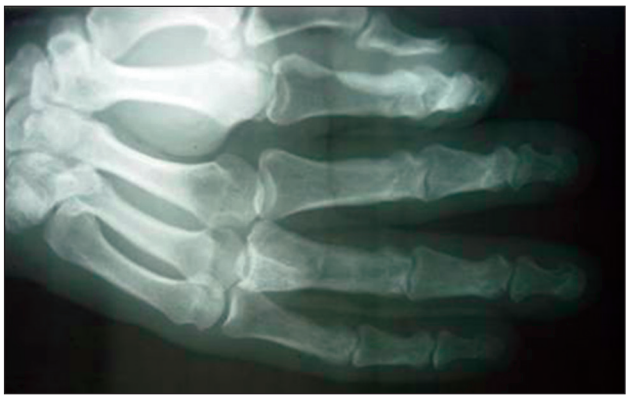
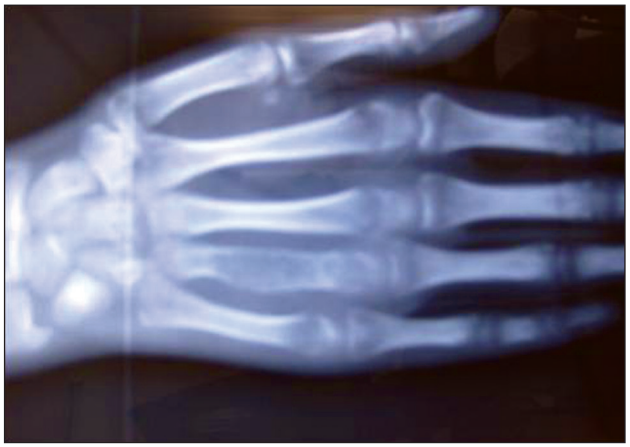


Figure 2. Postperative AP and Oblique graphy



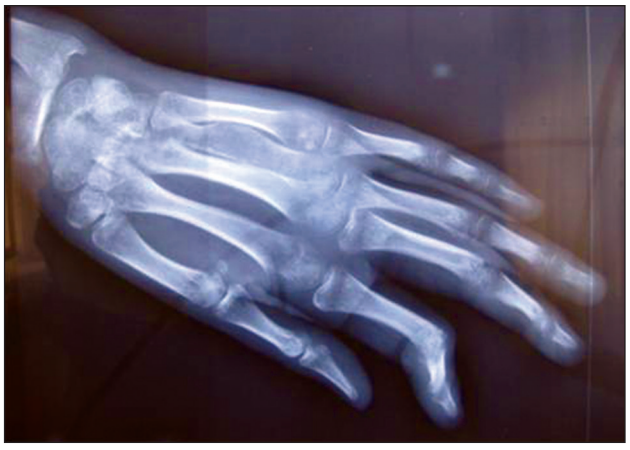


Figure 3. Preoperative AP and Oblique graphy

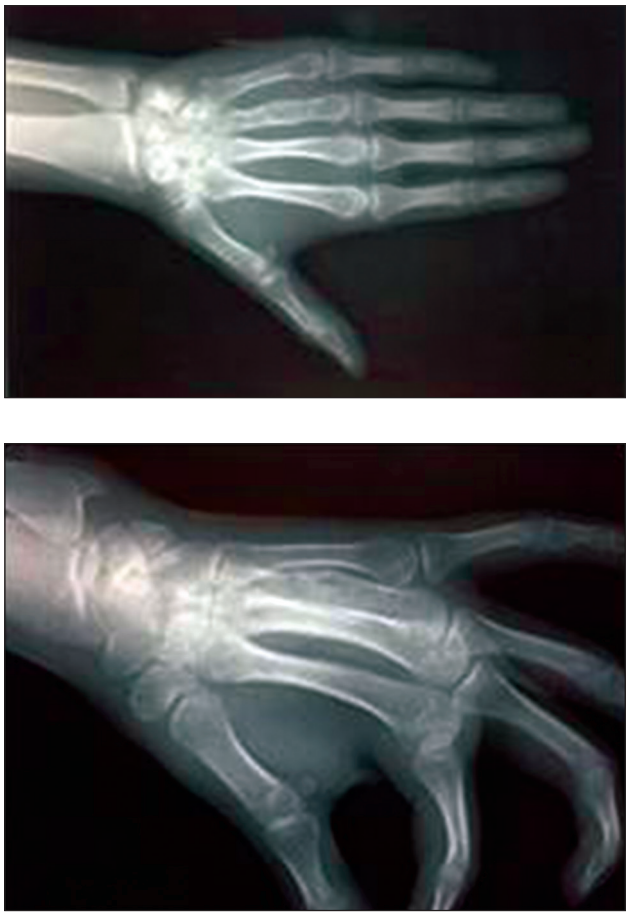


Figure 4. Postperative AP and Oblique graphy

Statistical analysis

SPSS for Windows 14.0 program was used for statistical analysis. Constant variables were expressed as mean ±standard deviation and categorical variables were expressed as percent. Chi-square test was used for assessment of non-parametric data and student's t-test was used for analysis of constant variables with normal distribution. A p level of <0.05 was accepted as statistically significant.

Discussion

The most common primary bone tumor of the hand and the wrist is enchondroma (20,21). The aim of treatment is to prevent malignant degeneration and to reduce likelihood of deformity and pathologic fracture that may develop following the destruction from the enlarging tumor. Verifying diagnosis of enchondroma through histologic examination of the surgical material is of important. Recurrence rate of enchondroma following curettage was reported as 2-15% and recurrence after treatment should make the surgeon to remember the likelihood of malignant histology. Solitary enchondromas carry the risk of malignant transformation in the ratio of 1% (22-26). The most common cause of admission of patients with enchondroma is pathologic fracture (6). The most common cause of admission is deformity and pain in our study, differently from literature. Only pain was the second leading cause of admission of our cases. Surgical intervention was postponed until healing of fracture was completed in 2 patients (6.5%) who admitted with pathologic fracture as complication risk increases if the fracture and the enchondroma are intervented concurrently.

Surgical treatment varies depending on tumor localization. Tumors involving small bones should certainly be treated due to the risk of pathologic fracture. Curettage and grafting or en-block bone resection may be performed in medullary located cases. Local recurrence may develop following incomplete resection (26-30). Using spongious morsalized autografts harvested from iliac crest, frozen-dried morsalized allografts or osteoconductive materials was recomended as grafting material after curettage (8-12). We preferred DBM as allograft in order to shorten operative time, to avoid general anesthesia and donor site complication.

Although enchondroma may be seen in all fingers, its localization in 4. and 5. fingers is more common (31). Megaro et al. reported that the tumor is localized in 4. and 5. fingers in the ratio of 50% (32). Localization of the masses included in our study is in parallel with literature and 48.3% of them were located in 4. and 5. fingers. In the study of Megaro et al. evaluating 38 patients, they reported that the tumor is seen in women in the ratio of 55.2% (32). This ratio was 61.3% in our study. Farzan et al. reported proximal phalanx involvement in 12 out of 19 cases that they detected enchondroma (33). Distal phalanx is less affected (14,34,35). Shimizu et al. detected that tumor was localized in distal phalanx in only 5 out of 47 patients that they operated due to enchondroma (5). In our study, while proximal phalanx was involved in 15 out of 31 cases, metacarp was involved in 8, distal phalanx was involved in 5, the less common localization of involvement was medial phalanx (3 cases).

There are also researchers who advocate that successful results are obtained with only curettage in solitary lesions (13-16). Kuur et al. (16) limited

the enchondromas which will undergo only curettage and reported that it should not be performed in only the excentric enchondromas. Hasselgren et al. (13) applied 1-3 week of immobilization after curettage in 28 patients and afterwards allowed to use the hand, they obtained moderate results in four patients, they did not encounter recurrence and fracture. Tordai et al. (14) performed only curettage in 44 patients with enchondroma and reported that one patient required operation due to recurrence. There are authors who recommend leaving the lesion site empty after endoscopic curettage (17). We used this method in none of our patients as only curettage procedure would render the lesion site unstable and precipitate pathologic fracture. Although filling the cavity with autograft or allograft do not contribute to stability much, we consider that it will be more effective than leaving it empty. Not only bone grafts but also hydroxyapatite and sterile casts were used to fill the cavity after curettage (11,12). Beer et al. (12) reported that bone integration was completed in 6-8 weeks in 22 patients that they applied hydroxyapatite after curettage. Hydroxyapatite application has become common recently in order to benefit from its osteoconductive effect (40).

Removal of tumor with curettage completely is important in prevention of recurrence. Recurrence rate is between 0-13.3% in literature (8-17). Recurrence was seen in only autograft group in our study and this ratio was 5.8%. in the cases that autograft was harvested from iliac crest, complications like bleeding, increase in duration of hospital stay, superficial infection, unwanted wound scar may be seen besides ilium fracture, chronic iliac crest pain, deep infection, abdominal herniation and also autografting requires general anesthesia (36-39). No severe complications except hypertrophic scar in two cases were seen in autograft group. Allografts have some disadvantages like late adaptation to graft site, immunologic rejection by the receiver, acting as viral infection carrier. Frozen-dried grafts have also the likelihood of carrying disease and immunologic rejection although infrequent (41). In our study, none of these complications were seen in allograft group and on the contrary to autograft group, local anesthesia use facilitated postoperative patient rehabilitation. Consolidation and duration of full hand grip strength's being close in autograft and allograft groups is striking. This may have arisen from DBM's having osteoconductive and osteoinductive effects. In the study of Bauer et al. delay in radiographically determined graft consolidation did not negatively affect clinical condition (10).

In conclusion, no difference is seen between success of DBM application as allograft and autografting. We consider that DBM use will increase as allograft as the result of reduction in allograft costs with developing technology and especially reduction in incorporation time with DBM.

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Bacterial contamination of the mobile phones of nursing students involved in direct patient care

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Abstract

The use of cell phones often occurs in hospitals, by patients, visitors and health care workers, and this is one environment where hospital-associated infection is most prevalent.The objective of this study was to determine the level and type of bacterial contamination of the mobile phones of nursing students involved in direct patient care. Samples from 40 nursing students's mobile phones were cultured and growth was identified using colony morphology, Gram stain, catalase and oxidase reaction. Also a questionnaire was used for data collection.

The rate of bacterial contamination of mobile phones is 47.5%. 52.63% of the isolates were identified as coagulase-negative staphylococci. Staphylococcus aureus strains were isolated from mobile phones of 31.58%. Gram negative bacilli were isolated from mobile phones of 15.79%.

According to these results it is obvious that, the training of students about disinfection is very important. Also, strict adherence to infection control and precautions such as hand washing and good hygienic practice among the users of mobile phones is advocated, to prevent the possibility of phones as vehicles of transmission of both hospital and community-acquired bacterial diseases.

Key words: Mobile phones, bacterial contamination, nursing students, phone hygiene**.**

Introduction

Today, mobile phones have become one of the indispensable accessories of professional and social life [1]. There is an international trend to incorporate mobile phones as well as other wireless technology to increase the efficiency, cost-effectiveness and quality of healthcare [2]. In Brady et al.'s study, 78% of HCWs expressed support for doctors' use of their mobile phone within the hospital environment; the approval ratings for nurses and patients were 56% and 49% respectively [3].

The use of cell phones often occurs in hospitals, by patients, visitors and health care workers, and this is one environment where hospital-associated infection is most prevalent [4]. Mobile phones act as perfect habitat for microbes to breed, especially in high temperature and humid conditions and may serve as vectors in transmitting nosocomial infections [5]. Brady et al. [3] had shown that the combination of constant handling and heat generated by the phones creates a prime breeding ground for microorganisms that are normally found in our skin. This may be because these types of bacteria increase in optimum temperature and phones are perfect for breeding these germs as they are kept warm and easy to handle in pockets, handbags and brief-cases [4].

The aim of present study was to determine the level and type of bacterial contamination of the mobile phones of nursing students involved in direct patient care.

Methods

The study was conducted in Sakarya University School of Health Sciences, in March 2011, with the participation of 40 third-grade volunteer nursing students during their clinical practice. Students were asked to participate in a self administered questionaire that developed by researchers. Age, gender, type of mobile phone of the students were entered in the questionnaire. Students were also asked to answer questions regarding cleaning of their mobile phone.

In total, forty mobile phones were surveyed. For sampling a sterile swab moistened with sterile saline was rotated over the surface of both sides including cover and key part of mobile phones. The sampling were immediately streaked onto two plates that consist of blood agar and eosin met-

hylene blue agar. Plates were incubated at 37°C for 24 h and 48 h. Isolated microorganisms were identified using colony morphology, Gram stain, catalase and oxidase reaction.

The bacteria which made beta-hemolysis, catalase positive, Gram-positive cocci seen under the microscope with Gram stain were defined acording to the the result of DNase and plasma coagulase test. Catalase negative, Gram-positive cocci were identified according to the microscopic appearance.

Isolated microorganisms from mobile phones were identified as Staphylococcus aureus, coagulase-negative staphylococci (CoNS) and Gram negative bacilli.

Results

The mean age of the participants was 20.48±1.48 (min 18- max 24). 67.5% (n=27) of them were female and 32.% (n=13) were male. Types of mobile phones 82.5% (n=33) were push-button and 17.5% (n=7) were touch screen. Study results about participants' mobil phone hygiene paractices are shown at Table 1.

The rate of bacterial contamination of mobile phones is 47.5%. 52.63% of the isolates were identified as coagulase-negative staphylococci. Staphylococcus aureus strains were isolated from mobile phones of 31.58%. Gram negative bacilli were isolated from mobile phones of 15.79%.

Discussion

In this study, 47.5% of mobile phones were found to be contaminated by bacterial agents. Colonization of potentially pathogenic organisms on mobile phones has been reported by Brady et al., Akinyemi, et al. Ulger et al., Borer et al., [3, 4, 6, 7].

Ulger et al., [6] investigated the contamination rate of the healthcare workers mobil phones and hands in opereting room and intensive care unit. The results of their study revealed a high percentage (94.5%) of bacterial contamination with different types of bacteria. Akinyemi, et al., [4] determined 15.3% bacterial contamination. In a study conducted by Ramesh, et al., [2] 45% of the samples were culture positive. Sadat-Ali et al., [8] found in their study that 43.6% health care providers carried infective organism on their cell phones, which could potentially cause infections. In a study that was conducted by Singh et al., [1] at a dental school in Manipal, India, in total, fifty mobile phones were cultured for microorganisms and %98 of them were culture-positive, and 34% grew potentially pathogenic bacteria. Kilic et al., [9] observed 61.3% growth in samples of mobile phones used by healthcare staffs in their study.

In another study conducted by Jayalakshmi et al., [10] except for the 12 new cellphones, all the others (91.6%) were found to be contaminated 76 (90.4%) owned by clinical doctors and 56(93.3%) owned by non clinical doctors. Srikanth et al., [11] sampled 51 mobile phones of healthcare workers

Table 1. Participants' practices about mobile phone hygiene

		n	%
Cleaning frequency of mobile phone	once a week	18	45.0
	biweekly	1	2.5
	monthly	9	22.5
	less than once per month	5	12.5
	never	7	17.5
What they use to clean their mobile phone?	wet wipes	30	75
	tissue paper	4	10
	cotton with alcohol	2	5.0
	cologne	3	7.5
	spray and brush	1	2.5
How they clean their mobile phone?	wiping the outer surface	37	92.5
	wiping the interspace of the key	1	2.5
	wiping by removing the key and cover parts	2	5

and 36 of corporate office workers. Among the mobile phones sampled, 94% were contaminated and only 6% were free of aerobic bacterial growth. In Saxena et al.'s study [12] 42% of mobile phones carried by HCWs and 18% carried by the general public were found to carry one or more organisms.

The most prevalent bacterial agent isolated from 52.63% of mobile phones was coagulase negative Staphylococcus (CNS). This result corroborates the findings of Singh et al., [1] (78%), Karabay et al. [13] (68.4%), Ramesh, et al. [2] (50%). Also in the Srikanth et al., [11] and Kilic et al.'s studies [9] the majority of isolates were Coagulase-negative Staphylococci. Akinyemi, et al., [4] found 26,3% and Saxena et al., [12] found 23% Coagulase-negative Staphylococci.

In our study, Staphylococcus aureus strains were isolated from mobile phones of 31.58%. Akinyemi, et al., [4] 36,8%, Singh et al., [1] 16%, Saxena et al., [12] 48%, Ulger, et al. 52%, [6] Sadat-Ali et al., [8] 33%, found Staphylococcus aureus in their studies. In Srikanth et al.,'s study meticillin resistant Staphylococcus aureus was 3.9%, meticillin sensitive Staphylococcus aureus was 7.8%.

In this study, other organisms isoleted inculed Gram negative bacilli. Gram negative bacilli were isolated from mobile phones of 15.79%. This was 17,68% in Srikanth et al.,'s study, 31,3% in Ulger, et al., 's study [6] , and 33% in Ramesh, et al.' s study [2].

Adequate decontamination of mobile communication devices is one approach which could reduce the risk of these devices in the cross-transmission of bacteria. Studies have consistently reported high numbers of staff who never clean their mobile communication devices (80-92%) [3,14,15] In our study it was determined that 17.5% of the students never clean and 12.5% of them clean only less than once per month their mobile phones.

According to these results it is obvious that, the training of students about disinfection is very important. Also, strict adherence to infection control and precautions such as hand washing and good hygienic practice among the users of mobile phones is advocated, to prevent the possibility of phones as vehicles of transmission of both hospital and community-acquired bacterial diseases [4].

Mobile phone producers should be aware and take action for designing of protective material against the bacterial contamination. Decontamination of mobile phones with alcohol disinfectant wipes may reduce the risk of cross contamination without any failure at device by using protective material. Furthermore, it is important for nursing education to comply hand-washing practices and routine surface disinfection through strict procedures to reduce nosocomial infections from crucial tool use such as mobile phones, pen etc.

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Seasonal change in the prevalence of cerebral venous thrombosis

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Abstract

Introduction: Cerebral venous thrombosis is rare and an uncommon cause of stroke. Unlike arterial stroke, cerebral venous thrombosis affects usually young adults. Seasonal effect on cerebral venous thrombosis has been rarely reported who is may be another risk factor of cerebral venous thrombosis. In this retrospective study, we try to investigate the seasonal change on frequency of sinus vein thrombosis in Southeast Turkey.

Material and methods: Between May 2008 and June 2011, Sixty two patients underwent CT venography or MR venography with clinical suspicion of cerebral venous thrombosis whose records were reviewed retrospectively.

Results: According to seasons most of the patients were applied to emergency service in autumn and according to the month most frequent patients were applied in January and November.

Conclusion: Seasonal change and climate may accept an independent risk factor for the cerebral venous thrombosis. If the patients apply to emergency services with headache and related symptoms in cold seasons, clinicians should be careful for cerebral venous thrombosis.

Key words: Cerebral veins, thrombosis, seasons.

Introduction

Cerebral venous thrombosis (CVT) is rare and an uncommon cause of stroke. Unlike arterial stroke, CVT affects usually young adults [1-4]. CVT can be presented with variable symptoms and clinical courses. CVT can lead to venous hypertension, edema, infarction, or hemorhages [5]. Most frequents patients admitted to emergency service with severe headache and seizures. The estimated annual incidence is 3 to 4 cases per 1 million population [4]. But the true incidence of CVT is unknown because of lack of adequate epidemiologic studies [6]. Females (75%) show much more propensity for CVT [4]. The different clinical presentations and lack of accurate diagnostic techniques are major problems for the diagnosis of CVT which may progress to death [7]. 7% of patients with CVT died in the acute stage of the disease [8]. Seasonal effect on CVT has been rarely reported who is may be another risk factor of CVT. In this retrospective study, we try to investigate the seasonal change on frequency of sinus vein thrombosis in Southeast Turkey.

Material and methods

Between May 2008 and June 2011, Sixty two patients underwent CT venography or MR venography with clinical suspicion of CVT whose records were reviewed retrospectively. The clinical symptoms of in patient with CVT were headache, vomiting, seizures, neurodeficits, visual defects disorientation, syncope in various combinations. Fifty two patients were undertaken the review that have had positive signs of CVT. The median age of the patients-34 women and 18 men-was 30.2 years (range, 1.5-65 years). All CT venography studies were performed on a CT with 64 detectors (Philips Brilliance CT scanner, Philips Medical Systems, Cleveland, Ohio). All MR venography were performed on Siemens Magnetom 1T MRI (Siemens, Erlangen, Germany) until December 2009, after this time MR venographies were performed on 3-T whole-body MR imager (Achieva; Philips Medical Systems, Best, The Netherlands)

CT scans were angled parallel to a line drawn from the posterior margin of the foramen magnum to the superior margin of the orbit to exclude the lens. If the patient was unconscious, scan was obtained without angled. In this circumstances an-

gle correction was supplied on workstation with multiplanar reconstructions (MPR). CT angiography scanning was performed with the following parameters: detector rows, 64; collimation, 0.625mm; pitch, 0.92; gantry rotation time, 0.75 s; slice thickness 0.90mm, slice increment, 0.45mm; 300mAs and 120 kV dose. A volume of 70-75mL of non-ionic contrast medium was injected at 4.0mL/s through an antecubital vein with an automatic power injector (Ulrich, Ulm, Germany). The scan delay time was 12 s determined by an automatic bolus-tracking program with a region of interest at the arcus aorta. The reconstructed data sets were sent to a workstation (Brilliance, Philips, Cleveland, USA) for post-processing to create MPR and maximum intensity projections (MIP). Rotation center MPR was also used to see all length of the dural sinus. Also we used routinely volume rendering display algorithms.

MRIs for the CVT were performed with conventional sequences (SE T1 weighted (W) and TSE T2W, T2 FLAIR) and Phased contrast venography. 3-T MRI acquisition parameters were: In the axial plane: TSE T2-weighted (TR/TE, 2500/80; slice thickness, 5 mm; interslice gap, 1 mm; matrix, 400 x 255; and NEX, 1) and FLAIR-weighted images (TR/TE/TI, 11000/120/2800ms; slice thickness, 5 mm; interslice gap, 1 mm; matrix, 256 x 148; and NEX, 1) and SE T1W images (TR/TE, 600/10 ms; slice thickness, 5mm; interslice gap 1 mm; matrix, 512x512; NEX, 1). 3D Phased Contrast Angiography (PCA), TR range/TE range, 19-20/7.4-7.9; flip angle, 10°; matrix size, 144 × 256; and PC velocity=10-30cm/sn. The total acquisition time was 10-12 minutes. The reconstructed data sets were sent to a workstation (Philips Extended Brilliance Workspace, Philips Medical Systems, Best, Netherlands) for post-processing and evaluation. After the acquisition of all source images of the MR venography sequence, the images were processed and displayed by means of an MIP algorithm using computer software. MR venography images (both source images, MIP images and conventional sequences) were analyzed for direct evidence of CVT, which included a lack of typical high-flow signal from a sinus that does not appear aplastic or hypoplastic on base images of MR venographic sequences. The indirect evidence of CVT included formation of collaterals over the extracranial veins, unusually prominent flow signal from deeper medullary veins, cerebral hemorrhage, visualization of emissary veins, and signs of increased intracranial pressure.

The exclusion criterion of the study was technically suboptimal CT or MR venography, which hindered proper visualization dural sinus thrombosis.

Results

19 patients were not taken to the review due to exclusion criteria. 52 patients have had positive sign of CVT. 34(65.4%) patient were female and 18(34.6%) were male. 3 (9.3%) patients were child. Major etiologies were gynecologic causes (include pregnancy and puerperium), chronic otitis (CO), idiopathic, cancer and factor V Leiden mutation.

Most frequent cause of CVT was gynecologic causes (36.5%). In this group, most frequent presentation months were September (15.7%), November (15.7%) and December (15.7%). The frequency of CVT presentations rates were 42.3%, 25.2%, 21% and 10.5% respectively in Autumn, Winter, Summer and Spring.

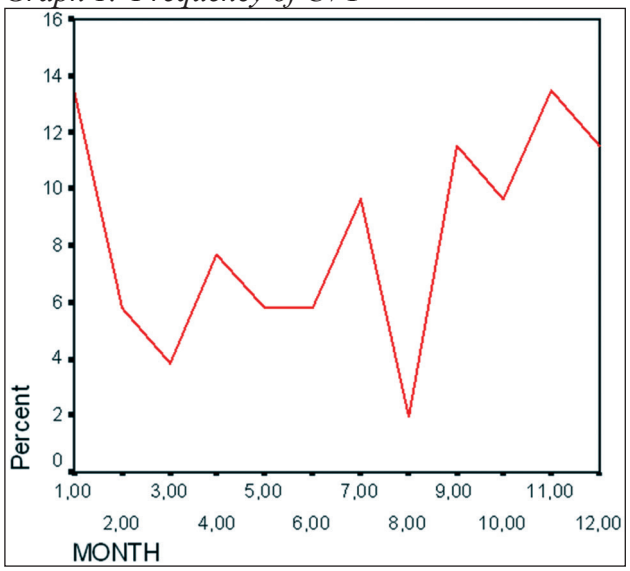
In this research, CO dependent CVT (32.7%) cases frequently occurred in January (17.6%), May (17.6%) and November (17.6%). The frequency of SVT presentations rates were 35.2%, 35.2%, 23.5% and 5.8% respectively in, Winter, Spring, Autumn and Summer.

In idiopathic group (23.7%), most commonly detected months were July (25%), January, September and October (16.6%). Seasonal rates for CVT were 41.1% autumn, 25% winter and summer, 8.3% spring in idiopathic group.

The other groups (cancer and Factor V mutation) whose frequency was 7.7%, distribution frequency was not able to given due to low patients count. When the CVT evaluated totally, it was seen most frequently in January (13.5%) and November (13.5%). In Graph 1, frequency of patient were shown according to the month. According to the season CVT was seen most frequently in the autumn (34.6%). Major involvement side of CVT was right sigmoid sinus (RSS) (Figure 1) that was seen 65.6%. RSS was seen more frequently with CO. As like RSS, left sigmoid sinus (LSS) involvement more frequently were seen due to CO. Right transverse sinus (RTS) and sagittal sinus (SS) involvement more frequently were seen in

idiopathic group (35%, 46.6% respectively). Left transverse sinus (LTV) (Figure 2) involvement more frequently was seen in gynecologic causes group (42%). As compared to right side to left side involvement, the ratio of Right/Left was 1.7.

Graph 1. Frequency of CVT



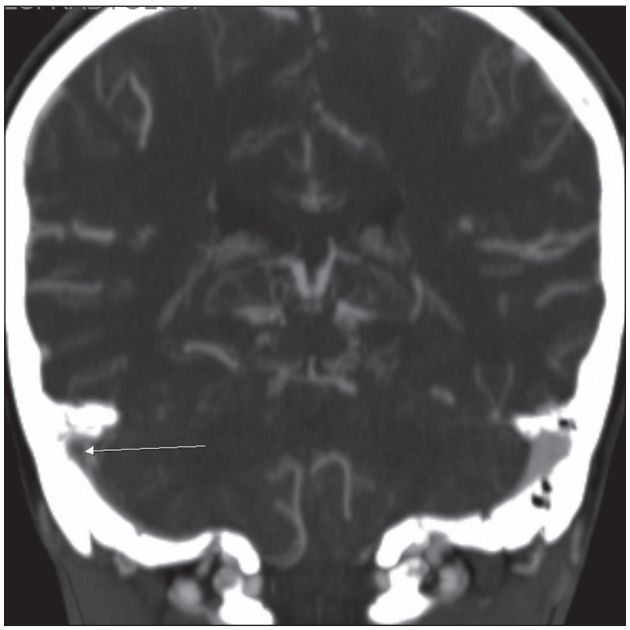


Figure 1. Coronal CT venography MIP image shows, right sigmoid sinus thrombosis (arrow)

The most common MR finding, seen in 83% of cases (43/52), was the presence on FLAIR–, T1-, and T2-weighted images, of a hyperintense signal in the dural sinuses, which is suggestive of intraluminal thrombus. Hemorrhagic infarcts were present in 36.5% (19/52) of patients (Figure 3).

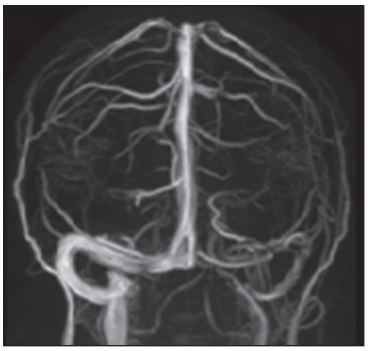


Figure 2. Maximum-intensity-projection MR venogram shows loss of flow signal in left transverse sinus

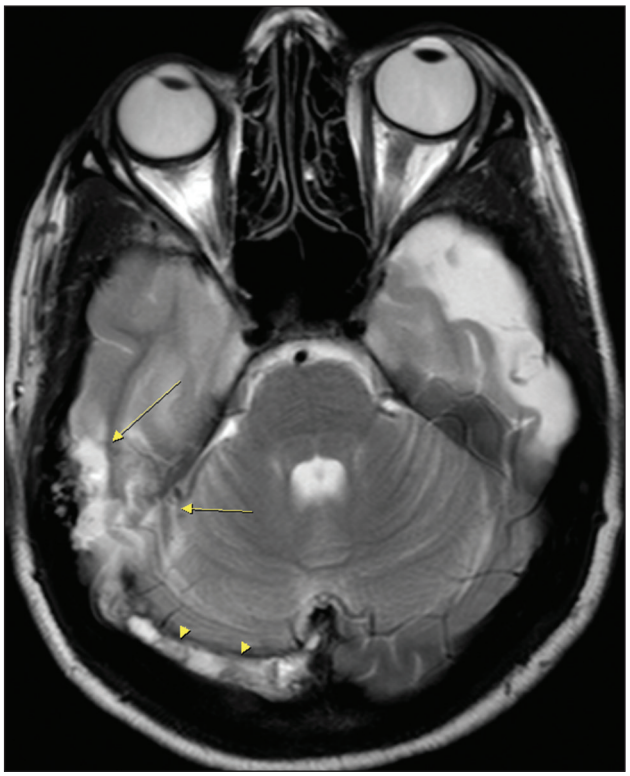


Figure 3. T2-weighted MR image in axial plane shows hyperintense signal in right transverse sinus and loss of normal flow void (arrow heads), and infarct in temporal lobe (arrows)

Discussion

Risk factors for CVT include inherited thrombophilia; factor V Leiden mutation, protein C and S deficiency, acquired prothrombotic state; pregnancy, purperium ,paroxysmal nocturnal hemoglobinuria, nephrotic syndrome, antiphospholipid antibodies, homocysteinemia, systemic disease; Behcet disease, systemic lupus erythematosus Wegener's granulomatosis, sarcoidosis, Inflammatory bowel

disease, neoplasia; leukemia, systemic carcinoma, systemic infectious disease, local causes (e.g. otitis, mastoiditis) and drugs; oral contraceptives, asparaginase, thalidomide, tamoxifen, erythropoietin, corticosteroids, sumatriptan etc. [9-11]. Also, CVT may constitute a complication of dehydration that can occur after common surgical procedures [12]. However even with extensive investigation, 20-25% cause of CVT cannot identified [13].

Diyarbakir is localized in southeast Turkey. General climate is dry. The highest temperature is seen in july and lowest temperature is seen January (Table 1).To the best of knowledge relationship between CVT and season was evaluated in a few study [14-16]. In our study CVT was seen most frequently in January and November and according to the season, CVT was seen most frequently in the autumn (34.6%) In compare to our results with Janghorbani et al. no difference were found to the seasonal changes [16]. However according to the month, they found September the most frequent in contrast to ours (January and November). Also Ferro et al. found higher number of CVT in autumn and October [14]. Slotz et al. found the most frequent season was winter, secondly summer [15]. Although not as high as Slotz et al. in our study, we found a peak in the july which months has the maximum temperature in southeast Turkey that may result in dehydration. When the cause related seasonal changes of CVT evaluated; gynecologic causes were found most frequent than other causes. Most frequent months were September, November, December and most frequent season was autumn. In Manfredinis' study, most frequent season of venous thromboembolism without CVT, had found autumn in gynecologic group [17]. In contrast to the Gynecologic group, in CO group most frequent months were January, May, November and most frequent season was winter. In our series, etiologies were not identified in 23.7% of the patients (Idiopathic group). In idiopathic group, as like GC and CO group, most frequent season was autumn, however interestingly july was most frequent month.

Generally pathophysiolgy of venous thromboembolism (VTE) had been reported about deep vein thrombosis (DVT) however it remains partially unexplained. In Öztuna et al. study, a statistically significant correlation between climates, seasonal change and case incidence of pulmonary embolism was demonstrated [18]. Also seasonal change in temperature is considered to be an important influential or causal factor for the seasonal patterning of cardiovascular mortality [17, 19]. Keatinge et al. demonstrated that the mild surface cooling increases platelet volume and count which may facilitate thrombosis [20]. A hypercoagulable state could be favored by elevated fibrinogen levels, which show a great seasonal variation, with a significant increase during the colder months [19]. Another prothrombotic risk factor is decreased physical activity which is limited, during colder temperature [19, 21]. Also the thermoregulatory arterio-venous shunt vasoconstriction may facilitate DVT by producing venous stasis and hypoxia [17, 19]. Additional to these factors Kalkanli et al. found factor V Leiden mutation prevalence 24.6 % in patient with deep vein thrombosis in Southeast Turkey [22]. In contrast to this result we found only 5.8% factor V Leiden mutation in the same region. Main difference between our and Kalkanlis' study is different localization of thrombosis. However due to lower number of patient, we couldn't analyze the seasonal change and factor V Leiden mutations relationship on CVT. In addition to above risk factor of CVT, in this study, some questions may be speculated. First, according to our study results, right side was more frequently involved than the left side. This involvement pattern did not change according to the causes (GC, CO and IP). In all patient evaluation of cerebral veins, no anatomic differentiation was

Table 1. 1975 – 2008 Mean Broadcast Information of Diyarbakir

Month	1	2	3	4	5	6	7	8	9	10	11	12
Mean Temperature (°C)	1.6	3.5	8.3	13.7	19.1	26.3	31.1	30.2	24.6	17.0	8.9	3.7
Mean maximum temperature (°C)	6.5	9.0	14.4	20.2	26.4	33.7	38.4	38.0	33.1	25.1	15.8	8.9
Mean minimum temperature (°C)	-2.5	-1.3	2.4	6.9	11.0	16.8	21.6	20.9	15.7	10.0	3.6	-0.4
Mean sunny hours	3.9	4.8	5.6	6.9	9.6	12.1	12.4	11.7	9.9	7.4	5.4	3.7
Mean rainy day	11.9	11.7	11.4	11.4	8.9	2.9	0.4	0.3	1.1	6.0	8.6	11.1

determined. Secondly, CO can be seen more frequent in autumn and winter than other seasons that may be expected due to increasing rate of infections in these seasons. However GC group was also involved most frequently in autumn too. Our hypothesis about these questions, first flow rate of cerebral veins may be decreased in fall and winter and right side may be more effected sinus. Secondly dominant hemisphere (generally left) blood circulation may be faster than the right side, so this slow rate may be an affective role on RSS.

Cerebral CT is used the first examination method in the diagnosis of CVT, because of it is easily available in most emergency services, and with the Multi Dedector CT (MDCT) technology it has short scanning time [9]. The diagnosis of CVT has traditionally been made with conventional angiography. MR imaging with MR venography is now commonly considered the noninvasive "gold standard" in diagnosing of the CVT. The combination of MR imaging showing the thrombosed vessel and MR venography demonstrating the non visualization of the same vessel is considered to be the best diagnostic tool in CVT [23, 24]. The most common MR finding, seen in 80% of cases, was the presence on proton density–, T1-, and T2-weighted images, of a hyperintense signal in the dural sinuses [7]. The use of a T2*-weighted gradient-echo (T2*GE) sequence very sensitive to all paramagnetic products of hemoglobin was also found particularly useful for early diagnosis of CVT [25]. However, MDCT angiography is an alternative diagnostic technique for researching CVT that is faster, more widely accessible, and more cost-effective than MR imaging [26].

In this study major limitation factor due to lower patient number in groups. Another limitation factor is that mildly higher number of patient in idiopathic group. Due to out of scope of this manuscript, imaging findings of CVT were not researched.

Conclusion

In conclusion, seasonal change and climate may accept an independent risk factor for the CVT. In southeast of Turkey, CVT is seen mostly in autumn and winter. If the patients apply to emergency services with headache and related symptoms in cold seasons, clinicians should be careful for CVT.

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Surgery and conservative treatment of giant cell granuloma of the maxilla - Case report

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Abstract

Introductory summary: Central giant cell granuloma (CGCG) is a benign but locally destructive lesion of the mandible or maxilla that occurs most often in the second and third decades of life. The clinical behavior of CGCG ranges from a slow-growing asymptomatic swelling to an aggressive lesion that presents with pain, local bone destruction, root resorption and tooth displacement. Therapeutic options have varied greatly over the years. Non-surgical treatments with alpha interferon (alpha-IFN), calcitonin and corticosteroids have been described and their benefits may be worthy of consideration. Surgery is considered a traditional treatment and it is still the most widely accepted, however in the literature not all authors agree on the type of surgery that should be performed.

Patients and methods: A case report of a giant cell granuloma treated with surgical and conservative treatment.

Results: The authors describe a successfully treated 11-year-old girl with an aggressive type CGCG lesion in the maxilla. Two weeks after the second surgical procedure, a conservative treatment was performed with 4 ml corticosteroids injection directly into the bone defect (protocol Terry – Jacoway (Triamcinolone acetonide 20mg/ml and Sol.Lidocaina 2% with epinephrine 1:200000 in relation 1:1) in duration of six weeks.

Conclusions: It can be concluded that both surgical and conservative procedures are equally important in the treatment of CGCG. In our opinion these procedures do not exclude each other but rather supplement each other.

Key words: Central giant cell granulomas (CGCG), reparative giant cell granuloma, craniofacial giant cell dysplasia.

Introduction

The central giant cell granuloma (CGCG) of the jaws is a well described clinical entity. However, etiology of this process is unknown and its biological behavior is poorly understood[1]. CGCG is an intraosseous lesion consisting of cellular fibrous tissue that contains multiple foci of hemorrhage, aggregations of multinucleated giant cells and occasionally trabeculae of woven bone[2]. CGCG, as described by Jaffe in 1953 is an idiopathic non-neoplastic proliferative lesion[3]. The term reparative giant cell granuloma was widely accepted at one time since CGCG was primarily considered as a local reparative reaction of bone, possibly to intramedullary hemorrhage or trauma. The use of the term reparative has subsequently been discontinued since the lesion represents essentially a destructive process[4]. CGCG affects females more often than males, in a 2:1 ratio and is seen most frequently under the age of 30 years[5]. Lesions occur more frequently in the mandible than in the maxilla. Lesions are more common in the anterior region of the jaws, and mandibular lesions frequently extend across the midline.

CGCG usually is an asymptomatic lesion, which may become evident during routine radiographic examination or as a result of painless but visible expansion of the affected jaw.

Aggressive and nonaggressive lesions have been described, with the likelihood of recurrence a feature of aggressive lesions.[6,7] In addition, the aggressive lesions are found in younger patients, grow quickly, cause pain and induce root resorption and bone perforation.[8] Studies have failed to identify any biochemical or histological differences between the aggressive and nonaggressive variants[9,10]. Most studies have looked for differences in giant cells to make such determinations, but no such differences have been found[11,12].

The management of CGCG will depend on the clinical and radiographic findings. Generally, curettage of well-defined localized lesions is associated with a low rate of recurrence. In extensive lesions with radiographic evidence of cortex perforation, a more radical excision is mandatory. In such cases even partial maxillectomy has to be done. The medical management of CGCG as an adjunct to surgery includes treatment with steroids or calcitonin[13] which inhibits osteoclastic activity. Some investigators subsequently reported successful treatment of CGCG with intralesional steroid injections.[14-17] Interferon-alpha appears useful in the management of aggressive CGCG, presumably due to its anti-angiogenic effects[18] Bisphosphonates have been administered intravenously in CGCG with promising results[19].

Case report

Patient MS, an 11-year-old girl, came to the Department of Maxillofacial Surgery, Faculty of Medicine, Niš, due to the swelling on the palate involving the upper jaw. The swelling has been present over the last three months with a constant tendency to increase followed by numbness in the area of the face on the left side, difficulty breathing through the left nostril and a sense of unease in the central and lateral incisors and left canine and premolars of the left upper jaw. History data did not show the existence of a systemic disease. Personal and family histories were without special features, with the absence of bad habits.

Extraoral testing did not indicate the existence of any pathological changes.

Regional lymph nodes were not enlarged.

Intraoral examination showed the presence of full dental arches in the upper jaw and good oral hygiene. All teeth in the upper jaw were vital. Clinical examination revealed a soft elastic increase in the hard palate, oval in shape with 40 mm diameter. Palpation was not painful, the area was elastic, with an unchanged surrounding mucous membrane, and a dark colored center (Figure 1, 2).

The following diagnostic tests were done: laboratory, ortopantomographic, puncture as well as multislice computerized tomography (MSCT).

The obtained laboratory results were within referent values, except for a parathyroid hormone (PTH) of 19.0 pg/ml, (normal range 8 – 76 pg/ml.).

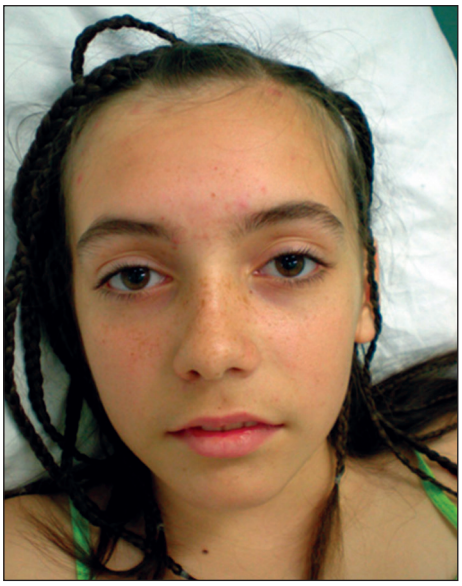


Figure 1. Patient at the beginning of the treatment

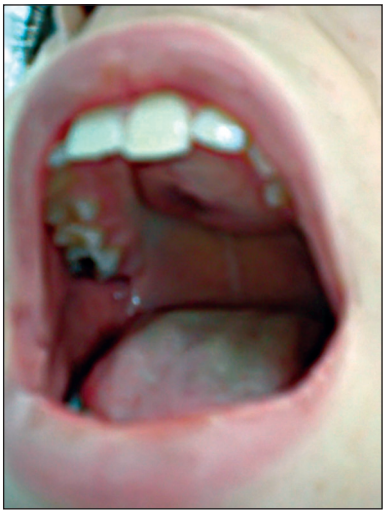


Figure 2. Intraoral examination at the beginning of the treatment

Radiological examination showed the existence of illuminated area of the upper jaw, centrally positioned, relatively clearly limited.

Puncture - after cytological analysis, the punctured material showed a lot of erythrocytes, leukocytes and macrophages, as well as numerous foreign body type giant cells and spindle-shaped proliferating connective-tissue cells.

Multislice CT scanner showed a lobulated tumor mass 25 X 35 X 35 mm (KkxLLxAP) in size, which had inhomogeneous peripheral post contrast enhancement. Tumor destroyed the hard palate and medial wall of the left maxillary sinus with the protrusion into the nasal cavity, oral cavity and left maxillary sinus (Figure 3a-c).

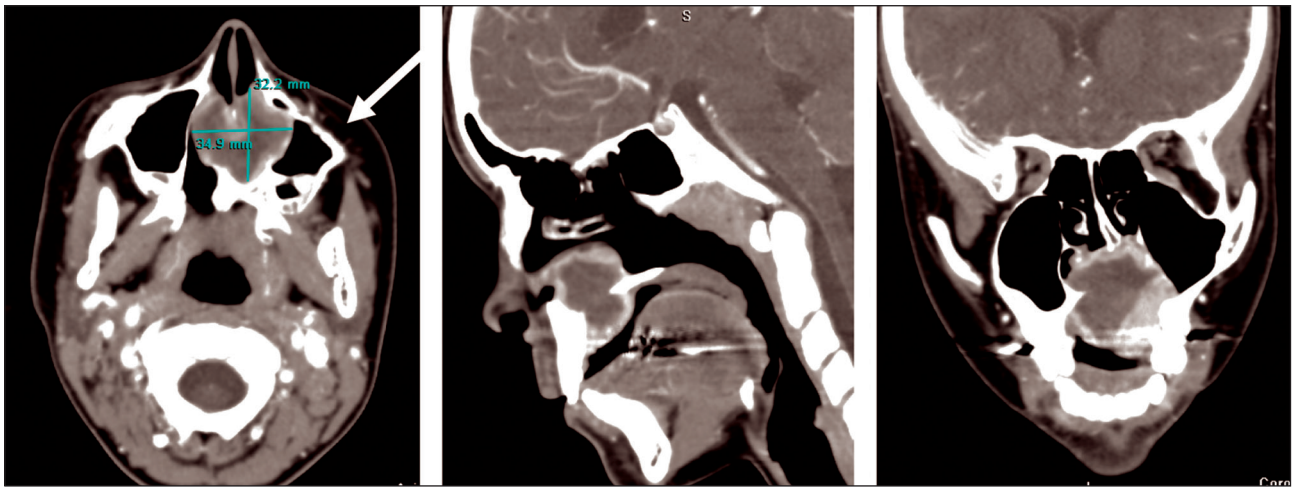
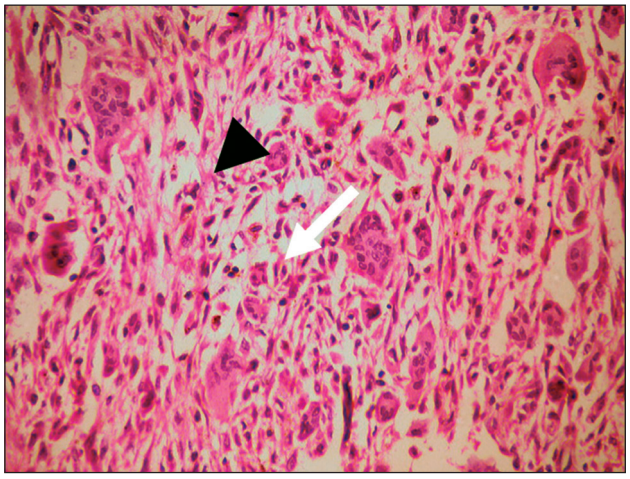


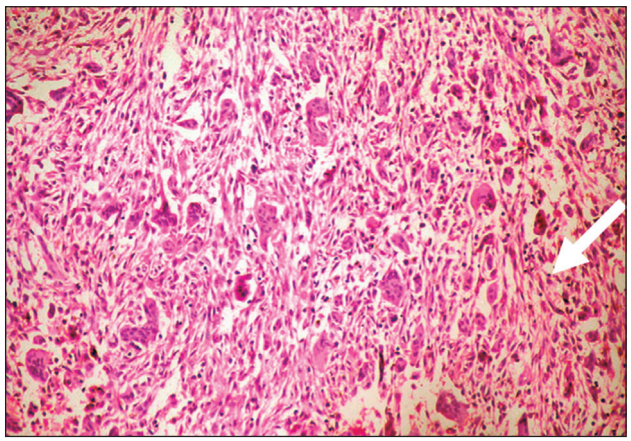
Figure 3a. Axial postcontrast MDCT scan shows hard palate destruction (arrow) caused by tumoral mass that has peripheral inhomogeneous postcontrast enhancement
Figure 3b. Sagittal postcontrast MDCT shows inhomogeneous tumoral mass at the level of hard palate in nasal cavity
Figure 3c. Coronal postcontrast MDCT scan demonstrates inhomogeneous tumoral mass in nasal cavity with destruction of nasal septum and maxillar alveolar ridge (arrow)

After all these diagnostic procedures, the surgical procedure was performed on May 13 2009. Using the intraoral approach, 2 mm of attached gingiva palatal teeth with the semi-circular section from the tooth 16 to tooth 26 width flap are raised using the existing defect on the hard palate. The following operation was performed: Extirpatio tumoris cum resectio partialis osis pallatini, septi nasi et conhae nasalis inferior lat.sin. Revisio sinus maxillaris lat sin sec Caldwell Luck.

The extirpated tissue was sent to histological verification (PH number 24892–97) and the obtained diagnosis was: Giant cell granuloma, aggressive form (Figure 4 a-b,5-6).



a)



b)

Figure 4a. and 4b. Numerous giant cells of foreign body type and fusiform connective tissue cells. 4a) HE, obj.x10 4b) HE, obj.x20.

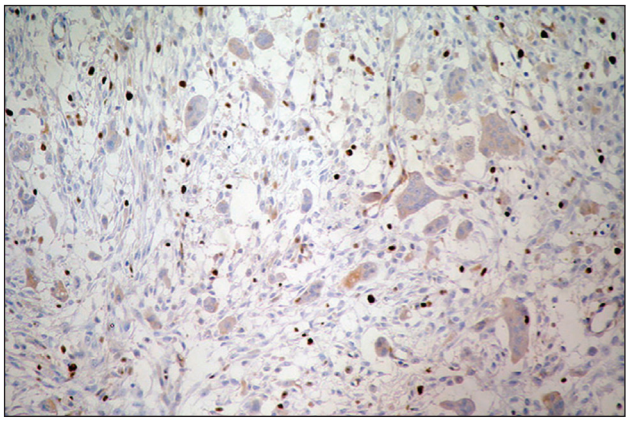


Figure 5. Giant cells were positive on CD68 (marker of macrofages. obj. x10.

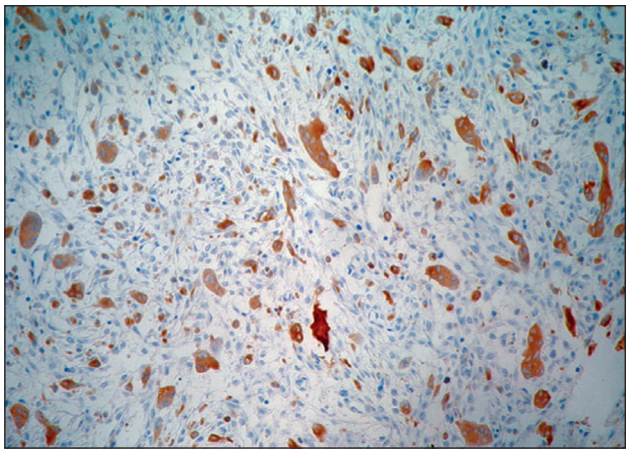


Figure 6. Ki67 activity was not detected in giant cell, but only in fusiform connective tissue cells. obj.x10.

Four months after the initial diagnosis and treatment, the lesion which was suspected to be recurrent, was noted by the control MSCT scans. Oval soft-tissue mass with 13x10mm diameter at the level of postoperative defect on the hard palate showed intensive and inhomogeneous post contrast enhancement. Left maxillary sinus with liquid content (Figure 7).

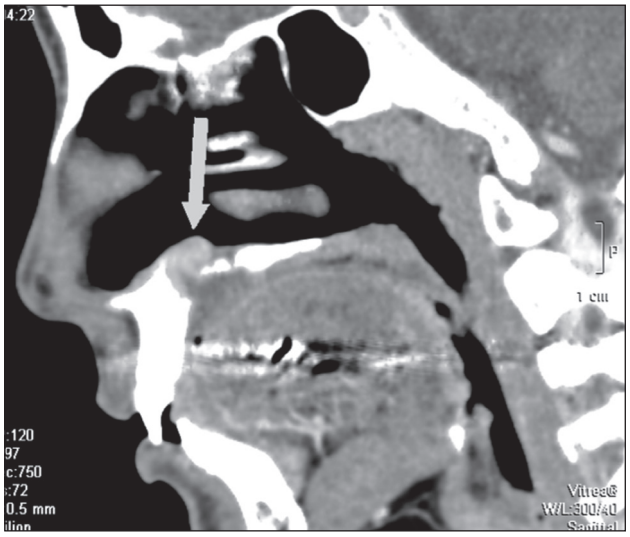


Figure 7. Sagittal postcontrast MDCT scan demonstrates recurrence with inhomogeneous postcontrast enhancement at the level of postoperative hard palate defect and maxillary alveolar ridge (arrow).

Another surgical procedure was performed by intraoral access above the upper vestibule of roots 13 to 25 (Figure 8) approaching the floor of the nasal cavity and it included: Extirpatio TU cavitis nasi. Revisio sinus maxilaris lat sin. PH number 62610–14 showed Dg: Chronic polypus sinusitis. Chronic rhinitis without recurrence. Postoperative recovery with antibiotic therapy (Amp. Galaceph 1 g in 24 hours,duration 5 days) passed normally.

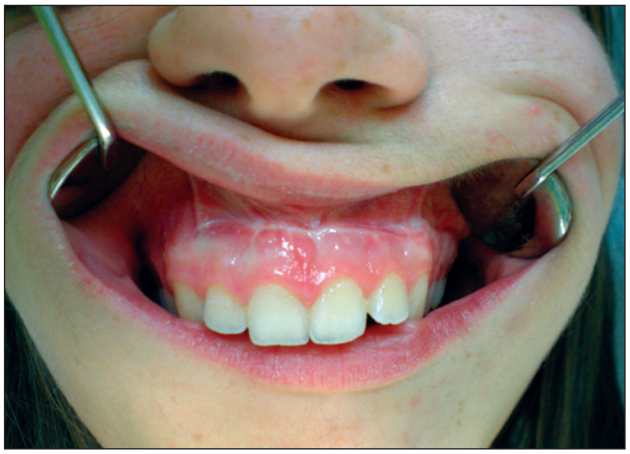


Figure 8. Intraoral examination after the second surgical procedure

After removing the tumor, a relatively well-defined cavity was obtained, as well as caudally limited palatal flap and the rest of the palatal bone, and cranially by mucous membrane of the nose floor and maxillary sinus bony walls. Since the bottom of the cavity is located below the level of cavum nasi and represents potential site for relapses, we decided to use corticosteroid injection according to the Terry–Jacoway[14] protocol. Our goal was to prevent possible relapses and induce formation of new bone (stimulating new bone formation) on palate, to prevent the creation of oronasal communication. It has been shown that osteoclast-like multinucleated giant cells decrease their lysosome protease extracellular production, which mediates bone resorption, in the presence of steroids[16]. Also, in animal experiments, steroids have been shown to induce apoptosis in osteoclasts[16]. These mechanisms result in a net decrease in bone resorption and a net increase in bone formation. Treatment was started with a 5 ml injection of Kenocort-A (10 mg/ml) and lidocaine solution 2% with epinephrine 1:200,000 50% mixture by volume). The solution (4 ml Kenacorte-A and lidocaine) was administered with a 5 cm disposable syringe with a 1 in # 22 G needle and injected intraorally through the front wall of the left maxilla in relation 1:1 ref.) over the next 6 weeks, starting

from 10 December 2009. After the third injection, it was observed that the amount of fluid required to fill the cavity was reduced.

The post-operative follow-up showed no adverse events and the control clinical examination, as well as CT, after one year were without changes (Figure 9,10,11a-b).

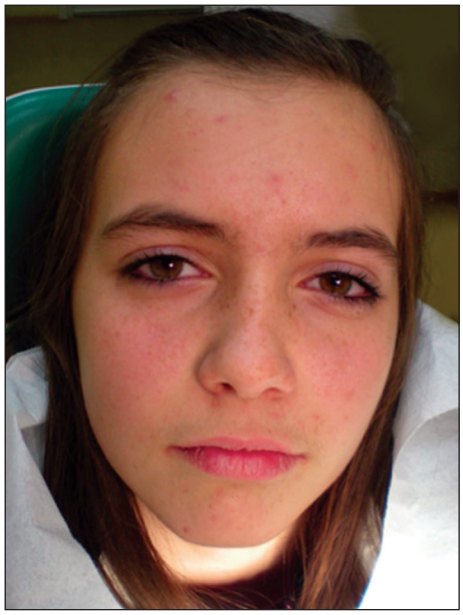


Figure 9. Patient at the end of the treatment.

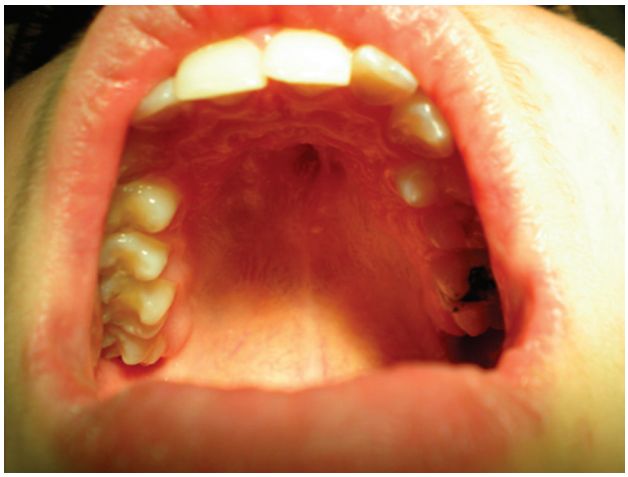
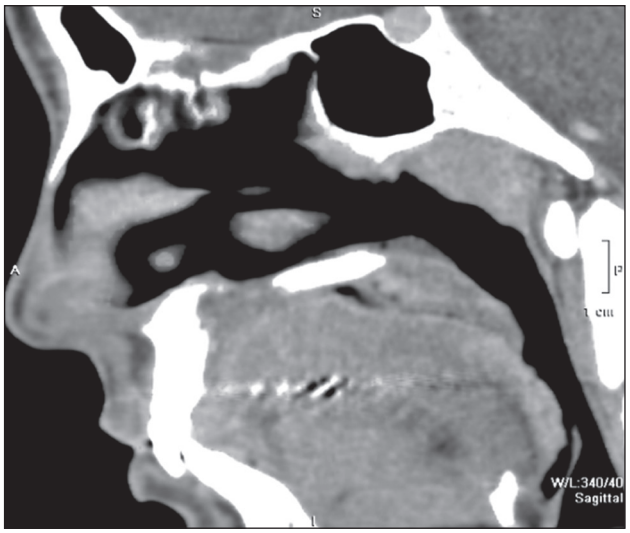


Figure 10. Intraoral examination at the end of the treatment



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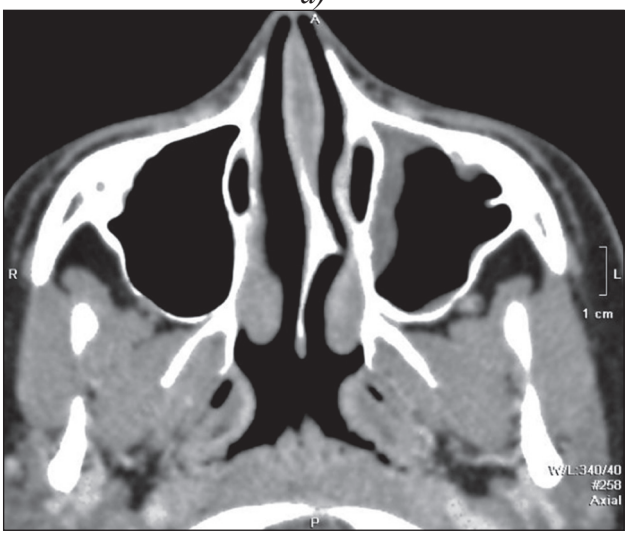

b)

Figure 11 a, b. Control postcontrast MSCT scans two years after the first surgery
a) sagittal
b) axial view shows normal findings and absence of recurrence. Sinusitis in the left maxillary sinus is present.

Discussion

CGCGs are benign but occasionally aggressive lesions[8]. CGCG commonly occurs in children and young adults with a slight predilection for females[20]. For now there is no right answer to the question of who is an etiological factor responsible for the occurrence of CGCG. Central giant cell granuloma remains a challenge for pathologists. There are multiple conditions that must be ruled out clinically, and the controversy surrounding the etiology of this condition has yet to be definitively resolved[21].

Many world pathologists[22] claim that central giant cell granuloma of the jaws remains an unsolved controversy, particularly regarding the question of whether it is a case of a reactive or neoplastic lesion or whether it is a question of aetiology. Because of the higher incidence of these lesions among girls and women, hormonal influences have been suggested as a possible causative factor in CGCG development[23]. Trauma has been considered as an important etiologic factor in the initiation of this lesion. The lesions increase by accumulation of tissue which is produced by slow, continuous hemorrhages of multicentric nature due to trauma and some defect in the capillaries[24]. The following should be considered in the differential diagnosis of primary central malignant lesions in this area: osteogenic sarcoma, fibrosarcoma, malignant fibrous histiocytoma, lymphoma and malignant giant cell tumor of bone[25].Our case represents a clinically aggressive, multifocal CGCG that is not associated with other conditions or syndromes.

Histopathology

According to electronmicroscopic and immunohistologic analysis, CGCG is a process that arises from monohistiocytelike cells[26].

CGCG lesions are composed of a hypercellular fibrous stroma containing numerous, multinucleated giant cells and often large sinusoidal spaces. The stroma typically contains abundant extravascular erythrocytes and hemosiderin[25]. Yamaguchi and Dorfman also proved that the aggressive variant of CGCG presented a high number of giant cells, an increased mitotic activity, and a high fractional surface area[27].

The treatment of small, nonaggressive CGCG lesions consists of thorough excision with careful curettage of the lesion. These lesions have a recurrence rate of 10 to 15 percent, whereas local aggressive giant cell lesions have a higher recurrence rate[28].

As the case report illustrates simple curettage of aggressive lesions is not appropriate because of their high recurrence rate. Several surgical methods have been suggested for removal of more aggressive CGCG lesions.

Radiation treatment, however, is contraindicated because of the potential for oncogenic potentiation[29]. Local injections of corticosteroids have also been suggested as means to treat any CGCG lesion[15].

Traditional treatment of CGCG has been performed by surgical removal of the lesion. Some authors recommended general resection including uninvolved bone; Chuong and colleagues have advocated an en bloc resection with immediate reconstruction of the affected area as the appropriate treatment for aggressive lesions[25]. The margins of the CGCG also may be thermally sterilized with a laser or cryoprobes[15] but some authors prefer conservative surgical treatment with curettage or curettage with peripheral ostectomy[8]. Resection of CGCG results in a major defect to the patient. This is of big importance, especially in children and young adults with developing dentition and jaws. Surgical treatment becomes even more difficult in patients with multiple lesions. In such cases, surgery may lead to extensive resection[26]. Radiotherapy has not proven to be a satisfactory alternative, because irradiation of giant-cell lesions may provoke malignant degradation[30].

Biopsy as well as histopatological confirmation of the lesion is obligatary before treatment with intralesional corticosteroid injections. Caution must be exercised with incisional biopsies, because the giant-cell lesion in bone has many clinical and radiographic similarities to a vascular lesion[26]. A careful medical history, auscultation, palpation and aspiration of the lesion before biopsy may prevent the problem of inadvertently entering a vascular lesion (with the possibility of uncontrollable hemorrhaging)[26].

The oral-maxillofacial surgeons must perform detailed evaluation of all patients to prevent any unwanted side effects of therapy.

Conclusion

Much controversy surrounds the CGCG. The main goal of this paper was to report the radiographic, histological and clinical features of CGCG. In addition, the surgical procedure in combination with the intralesional administration of corticosteroids was effective in treating central giant-cell granuloma, as well as in controlling the destructive and extensive nature of the disease.

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Anal canal carcinoma: Observetional study in a single center

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Abstract

Background: We aimed to assess our department's experience in treatment of anal canal cancer, to identify pathologic spectrum of the disease, and to evaluate the treatment modalities and outcomes in these patients.

Methods: Patients with anal canal malignancies treated at Department of General Surgery, Dicle University Medical Faculty, from 2005 to 2011, were retrospectively assessed. Medical records as well as histological examination results were all carefully registered and assessed.

Results: A total of 14 patients were treated for anal canal malignancies (except for anal melanomas) between 2005 and 2011. All patients were followed up for a period of 25 months (range, 5 months - 84 months). The patient group consisted of slightly more males than females (9 males, 5 females). The median age of the study population at presentation was 53.50±1.64 years (range, 22 - 77 years). Among 14 malignancies, 11 had squamous cell carcinomas (78.5%) while 3 had adenocarcinomas (21.5%).

Conclusion: Since signs and symptoms of malignant lesions resemble those of benign conditions, diagnosis and treatment pose a challenge. This condition, in addition to reluctance of many patients to get medical help for anorectal symptoms, may lead to false diagnoses and delay appropriate therapy.

Key words: Anal canal cancer, adenocarcinoma, Squamous cell carcinoma, anal canal, chemotherapy, radiation therapy

Introduction

Anal canal malignancies are rare neoplasms representing less than 5% of anorectal malignancies. They occur 20-30 times less commonly compared to colon carcinoma (1).

In United States, an estimated 5070 new cases of anal canal squamous cell carcinomas (SCC) presented in 2008, causing 680 deaths. Poor prognostic signs include a tumor size of > 2 cm and lymph node metastasis at initial diagnosis. The incidence of this malignancy is increasing in certain populations which practice high-risk sexual habits and have human immunodeficiency viral (HIV) infection. Human papillomavirus (HPV) infection and anal cancer association is strong and the former partly plays a role in the latter's pathogenesis (2).

Local control of the disease, sparing the anal sphincter, and avoiding a permanent colostomy are the current treatment aims of anal cancer therapy (3).

Until 1980, abdominoperineal resection was the preferred treatment for anal cancer, offering a reasonable 5-year survival rate of 60%. Unfortunately, there were significant perioperative morbidity and local recurrence rate of as high as 47% (2).

T and N stages constitute the most important prognostic factors for anal cancer. Tumor size and extent as well as nodal involvement have been established as important prognosticators in many studies over the last 25 years (4).

Later, three randomized trials have demonstrated that concurrent chemoradiotherapy with mitomycin and 5-FU was superior to radiotherapy alone in controlling local disease and improving survival rates. European Organization for Research and Treatment of Cancer (EORTC) also concluded in its study that concomitant 5-FU/mitomycin and radiotherapy was more beneficial than radiotherapy alone (3).

In this paper, we aimed to assess our department's experience in treatment of anal canal cancer, to identify pathologic spectrum of the disease, and to evaluate the treatment modalities and outcomes in these patients.

Patients and methods

We retrospectively reviewed patients with anal canal malignancies who were treated in the Department of General Surgery at the Dicle University Medical Faculty from 2005 to 2011.

Patient data was analyzed for the following variables: age, gender, presenting symptoms, mode of diagnosis, histological subtypes, stage of disease (according to Tumour, Nodes, Metastases [TNM] classification from the American Joint Committee on Cancer Manual for Staging of Cancer) and treatment received. Tumor assessments consisted of digital rectal examination, colonoscopy, and abdominopelvic CT scan. Rectal MRI and endorectal ultrasonography were performed as indicated.

Results

In the seven years spanning 2005 to 2011, we treated a total of 14 patients for anal canal malignancies (excluding anal melanomas). Table 1 shows a summary of the patient and tumour characteristics. Median follow-up period for all patients was 25 months (range, 5 months to 84 months).

Table 1. Patient and tumour characteristics

	No. (%)
Patient characteristics	
Total no.	14 (100)
Male	9 (64.2)
Female	5 (35.8)
Median; range (yrs)	53.5; 22-77
Median follow-up; range (mths)	25; 5-84
Tumour characteristics	
Histology	
Adenocarcinoma	3 (21.5)
Squamous cell carcinoma	11 (78.5)
TNM Stage	
1	1 (7.1)
2	3 (21.5)
3	9 (64.3)
4	1 (7.1)
Symptoms	
Anal pain	1 (7.1)
Bleeding	3 (21.5)
Anal pain + Bleeding	4 (28.6)
Mass	2 (14.2)
Mass + pain	2 (14.2)
Mass + Bleeding	2 (14.2)

TNM: Tumour, Node, Metastases

Among the 14 patients, there was a slight male predominance (9 male, 5 female). The median age at presentation was 53.50±1.64 years (range, 22 - 77 years). The malignancies were confirmed by biopsies, either during examination or during endoscopy (colonoscopy or sigmoidoscopy). The patients had the following disease histologies: 11 squamous cell carcinomas (78.5%), and 3 adenocarcinomas (21.5%). Up to half of patients had a combination of symptoms, most commonly presenting symptoms were anal pain and bleeding.

At the time of diagnosis, the majority of patients were in the advanced stages of the disease, i.e. TNM Stage 3 or 4 (71.4%). The type of treatment received varied, depending on the underlying histology, stage of disease and the overall health of the patient.

The patients either underwent surgical procedures, radiotherapy, chemoradiation therapy or a combination of treatment modalities. The types of surgical procedures performed are shown in Table 2**.**

Table 2. Primary Treatment Modalities according to Cell Types

Primary treatment Patients	(n =14)	%
Squamous		
Abdominoperineal Resection	3	21.5
Chemoradiotherapy	6	43
Palliative Radiotherapy	2	14.2
Adenocarinoma		
Abdominoperineal Resection	2	14.2
Chemoradiotherapy	1	7.1

Abdominopelvic resection followed by chemoradiation therapy was administered to two of three patients with adenocarcinoma, whereas the other patient underwent chemoradiation only.

Among 11 patients with SCC, 2 patients underwent palliative radiotherapy due to rectal bleeding, 3 patients underwent abdominoperineal resection followed by chemoradiation therapy, and 6 patients underwent chemoradiation only.

Discussion

The low incidence of anal canal malignancies compared to colorectal cancer has led to relatively fewer studies in literature on this disease. Our study presents 14 patients who constituted 2.9% of all anorectal tumors treated during the re-

viewed time period, which is consistent with the current estimate of 1-5% in the literature. Anal canal tumors typically present in the seventh decade with a prominent female predominance, with a female-male ratio of approximately five to one. Our study group had a median age of 53.5 years (range 22–77) at presentation, with almost similar gender distribution (9 males and 5 females) (1, 5-7). SCC is the most common histological subtype of anal canal malignancies, with a frequency of 80% (8). Cloagenic, basaloid, and transitional tumors are its variants having similar natural history, response to treatment, and prognosis (3, 9). Adenocarcinomas make up 15% of anal canal tumors and the remaining are rarer subtypes such as melanomas, leiomyosarcomas, and carcinoid tumors (3, 9-11). Our series had a subtype distribution similar to previous reports, approximately 21.3% of the cases were adenocarcinomas while 78.7% were squamous cell carcinomas.

Most of the patients (70%–80%) usually have non-specific symptoms (9). Accurate diagnosis remains a challenge as benign conditions such as haemorrhoids, fissures, fistulae, leukoplakia and Paget's disease may coexist. Patients usually present with combinations of the most common symptoms, i.e. bright red rectal hemorrhage, pain, anal discharge, and pruritus. More advanced disease is suggested by rarer symptoms of incontinence, fistulation, and pelvic pain (1,9). Our patients also presented most commonly with rectal bleeding (64.3%) and pain (49.9%), with 57% of the patients presenting with both.

Thanks to the distal location of anal canal cancers, clinical evaluation is straightforward. Hence, a complete examination should include digital rectal examination, anal proctoscopy, and palpation of inguinal lymph nodes. Such an initial examination permits to estimate the size and fixity of the tumor. Histological confirmation can be accomplished by transanal biopsy. Endoanal ultrasonography, though a highly operator-dependent procedure, is performed to have further information on the depth of the lesion, sphincter complex involvement, and perirectal lymphadenopathy. Computed tomography and magnetic resonance imaging have a role in final staging, in addition to certain situations where ultrasonography is too uncomfortable due to a stenotic or bulky lesion. Anal canal cancer rarely (10%) metastasizes to distant sites. Inguinal or the pelvic lymph nodes may be involved, with overall inguinal lymph node involvement being 10%–20% (1, 9). Our study group exhibited a distant metastasis incidence of only 7.1% (TNM Stage 4).

Majority of anal canal tumors are SCC. Until the revolutionary work by Nigro et al. in the 1970s (12), APR and permanent colostomy were major therapeutic interventions, with five-year survival rates and recurrence rates of 38%–71% and 27%–43%.(5-7), respectively.

Chemoraditation introduced later as the primary treatment reached similar survival and recurrence rates to surgery; furthermore, they had an added advantage of sphincter preservation in up to 80% of patients (13). As a result of three large randomized controlled trials (14-16), the current recommendation for anal cancer treatment is to employ 5-fluorouracil (with at least 45-Gy) and mitomycin together with radiation; the latter generally should include inguinal lymph node basin. This practice has allowed five-year survival and recurrence rates of 58%–90% and 7%–19%, respectively (17). Surgery is currently indicated for incomplete response to chemoradiation or recurrent disease in the form of salvage surgery. A total of 5 patients with SCC (21.5%) and adenocarcinoma (14.2%) in our series underwent APR, due to local recurrence and unresponsiveness to CRT.

Despite continuing efforts to define new prognostic tools for anal cancer, T and N classification are the most commonly used and reliable prognostic factors. As indicated by some studies but unconfirmed by others, women might have a better prognosis compared to men. Histologic subtypes and degree of differentiation do not seem to have a clear effect on prognosis; furthermore, subtypes and grades are not determined by uniform criteria and with reproducibility. An important prognostic factor is the response to radiotherapy which could be used as a marker for tumor biology. Dose and treatment time of radiotherapy appear to have prognostic importance (4).

Until 1980, APR was the standard treatment (18); however, as the CRT has been accepted as the "gold standard", the treatment has achieved excellent short- and long-term outcomes; in addition, it has improved quality of life by eliminating

need for extensive surgeries as well as adverse effects of high dose radiotherapy (19 ,20).

A considerable majority of patients benefited higher local control rates and enjoyed anal sphincter activity sparing with CRT. Thus, surgery has fallen to secondary position in treatment, recommended only to patients without a complete response to the primary therapy. Anal cancer is a rare and complex disease requiring close cooperation between various disciplines which are the keys for appropriate and adequate patient care. Anal cancer screening and efforts for prevention are increasingly appreciated in clinical practice; however, further studies are clearly needed (3, 19, 21).

Conclusion

Anal canal cancer is a rare malignancy of the gastrointestinal system. Since its signs and symptoms resemble those of various benign conditions, diagnosis and management pose a challenge. This diagnostic ambiguity coupled with hesitancy of many patients to see a physician for anorectal symptoms are potential contributors for misdiagnosis, and diagnosis and treatment delays. Most patients may have favorable outcomes, by means of early diagnosis and appropriate oncologic directed therapy.

Conflict of interest

The authors have no conflicts of interest or competing financial interests with regards to this manuscript.

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The use of concentrate growth factors in Gyded bone regeneration after lateral sinus lift procedure (case report)

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Abstract

Introduction: Elevation of the sinus floor to increase the alveolar bone needed to place implants is considered a highly predictable and effective treatment option. Many techniques have been described to achieve vertical augmentation of the maxillary sinus mucosa. One of the very good and practical solutions is application of Concentrated Growth Factors (CGF) alone or mixed with bone graft. Growth factors are proteins, which regulate the complex processes of wound healing. Growth factors play a main role in cell migration, cell proliferation and angiogenesis in tissue regeneration phase.

Case report: In this paper, we described a case of a 52-year old female patient, who reported to the Department of Oral Implantology of the Clinic for Dentistry of Vojvodina for prosthetic restoration of toothless upper left jaw. After obtaining an informed consent from the patient, lateral sinus lift was performed along with the augmentation of the missing bone, using concentrated growth factors (CGF) mixed with alloplastic material (BioOss). The preservation of the augmented region was performed using the BioGuide membrane. At the same time, two conventional implants (Nobel) with 13 mm length and 4.3 mm diameter were placed.

Conclusion: Application of fibrin rich *block* with *concentrated growth factors* (CGF) is one of the latest approaches to guided bone regeneration. The method is relatively simple, without risk of transmissible and allergic diseases and economically feasible.

Key words: Sinus lift, growth factors, bone regeneration.

Introduction

Elevation of the sinus floor to increase the alveolar bone needed to place implants is considered a highly predictable and effective treatment option. (1-3)

Many techniques have been described to achieve vertical augmentation of the maxillary sinus mucosa. When considering a lateral approach to the sinus, the major differences between the various surgeries consist of the type of grafting material used and the decision of immediate or delayed implant placement. One of the very good and practical solutions is application of concentrated growth factors (CGF) alone or mixed with bone graft. (4)

Historical background of platelet aggregation

Growth factors are proteins, which regulate in the complex processes of wound healing. Growth factors play a main role in cell migration, cell proliferation and angiogenesis in tissue regeneration phase. (5)

These growth factors are mainly located in blood plasma and platelets. Platelet aggregate has been widely used to accelerate tissue regeneration and repair in dental and medical area. As a first generation of platelet concentrate, platelet rich plasma (PRP) and platelet rich in growth factor (PRGF) have been widely known. PRP was first introduced by Marx. (6)

However, the hard-tissue regenerative effects of PRP proved poor and controversial. (7)

As second generation of platelet aggregation, platelet-rich fibrin (PRF) was developed by Choukroun. (8)

PRF is a fibrin-rich gel produced with fresh venous blood taken from a patient's vein. The PRF protocol is simple and predictable. Patient's veno-

us blood sample is taken without anticoagulant in 10-mL tubes, which are immediately centrifuged at 3000 rpm (approximately 400g according to our calculations) for 10 minutes. After 10 minutes centrifugation, a fibrin-rich gel with aggregated platelet is obtained in the middle of the tube, just between the red corpuscles at the bottom and platelet poor plasma at the top. The fibrin-rich gel accelerates the formation of new bone and soft tissue healing as a result of growth factor release. (9-10)

PRF protocol requires neither biochemical additives (such as bovine thrombin) nor chemical additives (such as Calcium Chloride) to induce jellification. Thus, PRF is a safe procedure with no concern of cross-contamination. Concentrated growth factors (CGF) were first developed by Sacco. (11)

CGF is produced by the centrifugation of venous blood as same as PRF. However, the technique is different on centrifugation speed. Unlike PRF, CGF use variable rpm from 2400-2700 rpm to separate cells in the venous blood, therefore, results in fibrin rich blocks that are much larger, denser and richer in GF than common PRF. This shows better regenerative capacity and higher versatility when using the fibrin rich block. According to Professor Rodella from the University of Brescia, Department of Biomedical Sciences and Biotechnologies, CGF shows higher tensile strength, more growth factors, higher viscosity and higher adhesive strength than PRF. Thus, surgeons can use CGF as barrier membrane to accelerate soft tissue healing or mix it with bone graft to accelerate new bone formation. (12)

Case report

In this paper, we described a case of a 52-year old female patient, who reported to the Department of Oral Implantology of the Clinic for Dentistry of Vojvodina for prosthetic restoration of the toothless upper left jaw. (Fig. 1)

Following clinical examination and radiograph analysis, placement of two endoosseous titanium implants was suggested as an option to replace the missing teeth in regions 24 and 26. With an aim of more precise procedure planning, 3D Cone Beam scan of the region was performed, revealing vertical bone loss on tooth 26 and the risk of potential perforation of sinus mucosa by the implant.

After obtaining a consent from the patient, we decided to perform lateral sinus lift along with the augmentation of the missing bone using concentrated growth factors (CGF) mixed with alloplastic material (BioOss). Application of BioGuide membrane was planned to preserve of the augmented region. Concomitant placement of two conventional implants (Nobel) with 13 mm length and 4.3 mm diameter was planned, also.

Preparation of the fibrin block with concentrated growth factors was the initial step of the procedure. Patient's blood sample (40 ml) was taken directly from the vein, without anticoagulant, and distributed into four sterile 10-ml tubes. The tubes were centrifuged for 14 minutes at 2500 rpm. After centrifugation, the tubes were placed onto the tube holder and fragmentation was immediately visible (Fig. 2). The upper layer contained the separated serum, fibrin rich block with concentrated growth factors is obtained in the middle of the tube whereas blood corpuscles precipitated in the bottom layer (Fig. 3). After pouring off the serum, the middle and lower layers were carefully harvested from the tubes using a pean forceps, and placed into the sterile glass container. The lower (red) layer was removed with scissors. Thus we obtained the pure middle layer, i.e. fibrin block rich in concentrated growth factors, which was ready to be used for filling the sinus cavity (Fig. 4 and 5).

After preparing the concentrated growth factors (CGF) the surgical sinus lift procedure was performed using a lateral window approach. The procedure involved creation of lateral bony window in the anterior maxillary wall in the region of missing tooth 26. The exposed sinus mucosa was carefully detached from the bony walls and displaced cranially, taking care to prevent its perforation. The space between bony sinus walls and sinus mucosa was filled with three fibrin blocks reach with concentrated growth factors (Fig. 6), which were previously mixed with alloplastic material (BioOss). Immediately, two conventional (bone level) implants with 13 mm length and 4.3 mm diameter were placed, taking care to prevent injury and perforation of sinus mucosa. After implantation, lateral bone defect of maxillary sinus was covered with the fourth fibrin block that was pressed to form a fibrin membrane. The entire augmented and implanted region was finally covered

with resorptive BioGuide membrane to prevent proliferation of the soft tissue and compromising of guided bone regeneration (Figs. 7, 8, 9).

Mucoperiosteal flap was relaxed by making the incision in the periosteum to prevent its tension, and sutured back in place combining vertical mattress suture and a single suture (Fig. 10)

After completed surgical procedure, full reparation and regeneration of the bone should be achieved within a four-month period enabling further prosthetic rehabilitation of the patient.

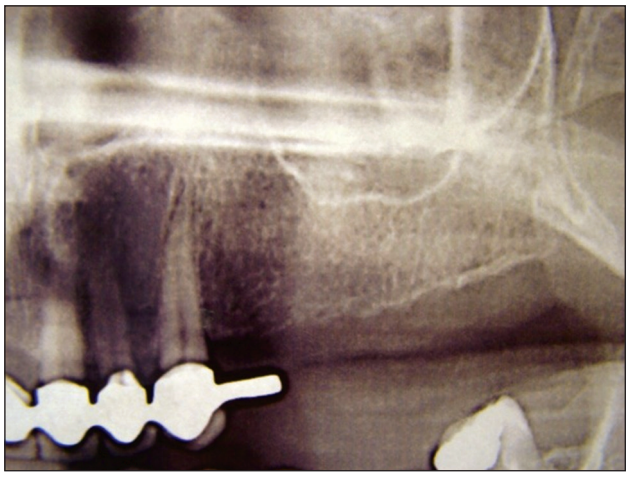

Figure 1. Pre-operative OPT scan

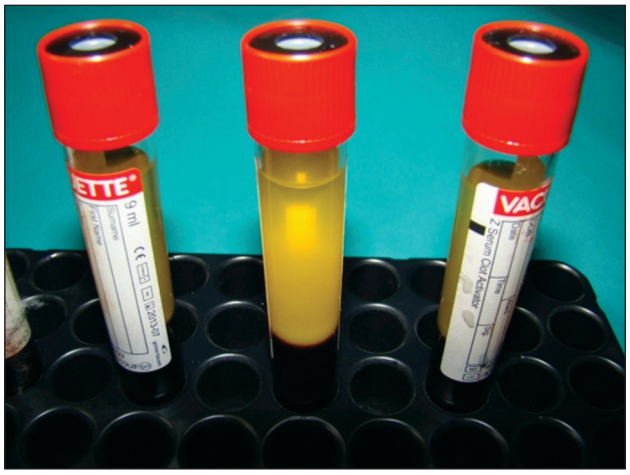

Figure 2. Status after centrifugation

Figure 3. Separated blood fractions

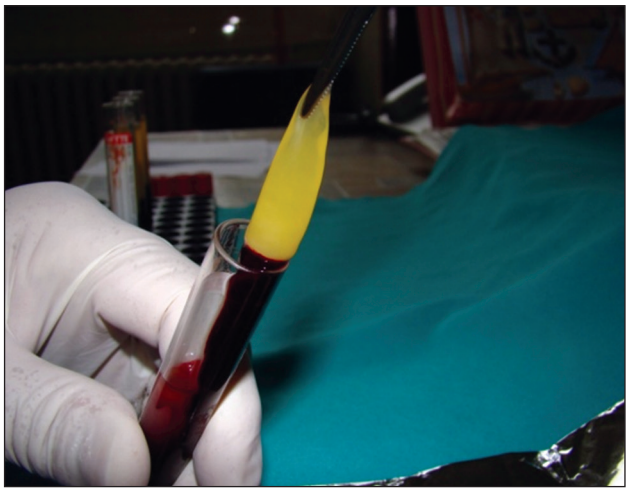

Figure 4. Qualitative appearance of the preparation

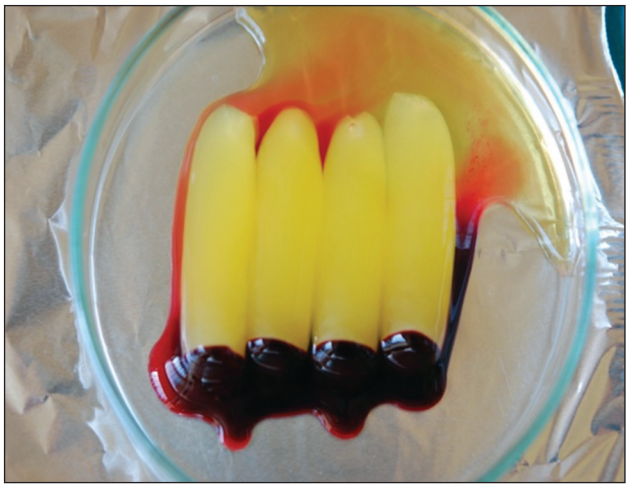

Figure 5. Prepared fibrin blocks, ready for use

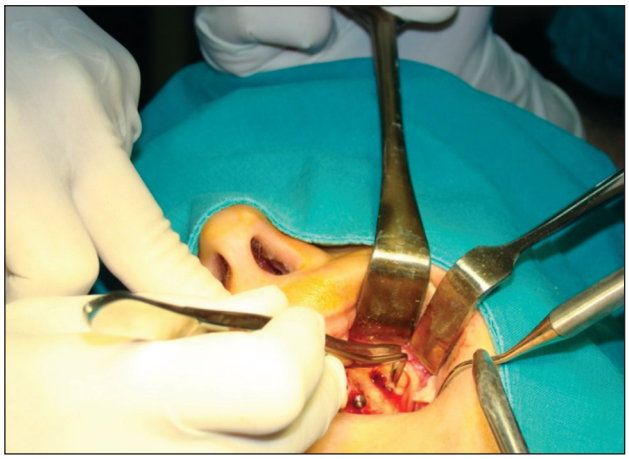

Figure 6. Filling the sinus cavity

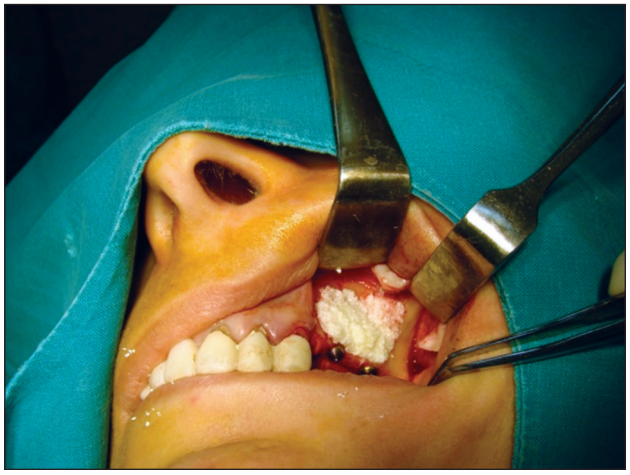

Figure 7. Covering the defect with allograft

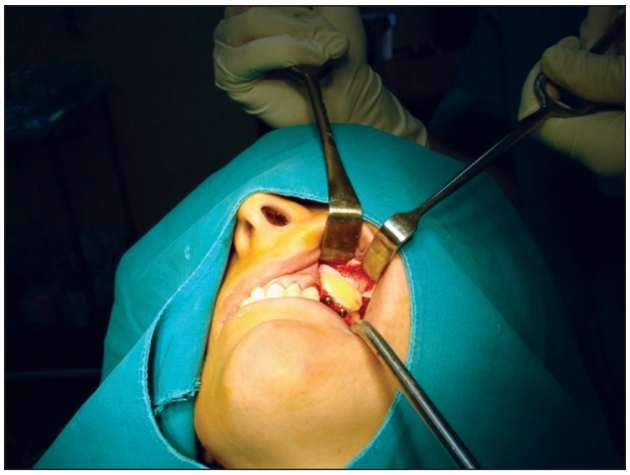

Figure 8. Allograft covered with CGF

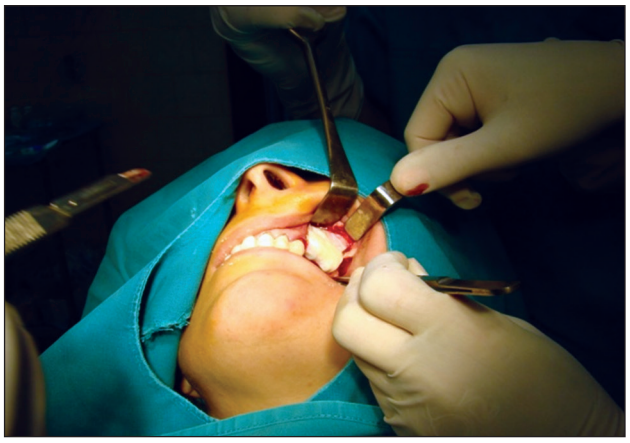

Figure 9. Placed BioGuide membrane

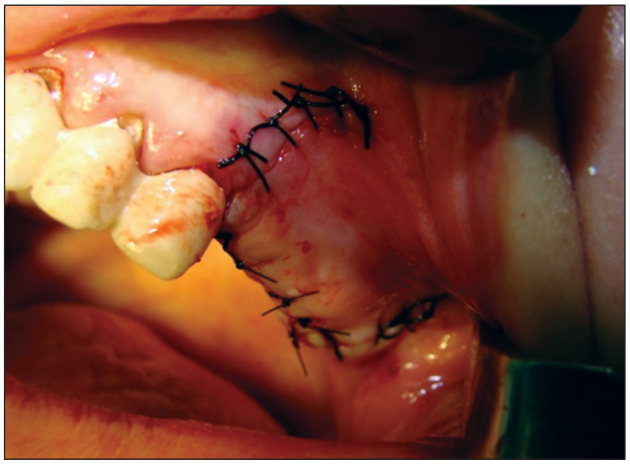

Figure 10. Final appearance of wound

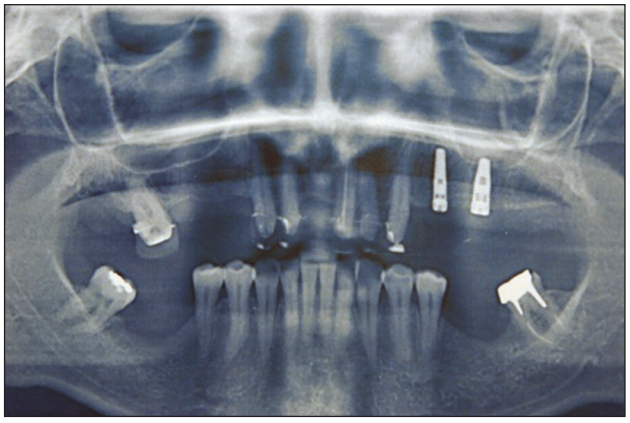

Figure 11. Control RTG

Conclusion

Application of fibrin rich *block* with *concentrated growth factors* (CGF) is one of the latest approaches to guided bone regeneration. Concentrated growth factors are applicable in all bone defects of the dento-alveolar region, with an aim of preventing *disturbance* of *bone* and *soft* tissue *architecture*. The method is relatively simple, cost effective and without risk of transmissible and allergic diseases.

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An analysis of dermatology journals currently published around the World: An internet-based preliminary study

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Abstract

Background: The medical literature is lacking a comprehensive evaluation of dermatology journals actively publishing around the world.

Objective: This study was conducted to identify all dermatology journals around the world actively publishing in November 2011 and evaluate their features such as origin country, publication frequency and accessibility.

Methods: Currently published dermatology journals were searched on five international medical databases including PubMed/MEDLINE, Science Citation Index, Science Citation Index Expanded, Index Copernicus and Directory of Open Access Journals. Websites of the journals were investigated and a list of actively referenced periodicals was compiled.

Results: Ninety-seven dermatology journals were identified, published in 28 countries. Pubmed was the largest database with 67 dermatology periodicals. 32 journals were published in the USA and 11 were in the UK. The mean age of journals was 30.21 ± 3.32 years (range 1–223). The oldest dermatology journal of the world was 223-year-old *Dermatology* from Switzerland. Most journals (36 %) were published bimonthly.

Conclusion: Our preliminary study confirms that dermatology publishing is not homogenous worldwide. Further studies investigating national databases may be needed.

Key words: Dermatology, journal, publications, dermatology literature, citation analysis.

Introduction

Dermatology *is a fast-growing* and dynamic *branch of medical* science with newer drugs and procedures being applied to improve the physical appearance of patients. Particularly in developing countries, scientists feel increasing pressure to publish their articles in peer-reviewed journals. Today the medical journals are easily accessible through the Internet. Impact factor is considered to be the main tool in assessing the quality of a publication and for a journal it is vital to be indexed by major databases.

The objective of this study is to provide an overview of actively indexed dermatology journals in five international popular databases and grouped them by publication frequency, origin country and accessibility.

Methods

Dermatology periodicals were searched in five international databases which permit a public and free search in their master journal lists. The search was carried out in the databases including Pubmed/Medline, Science Citation Index (SCI), Science Citation Index Expanded (SCI-E), Index Copernicus (IC) and Directory of Open Access Journals (DOAJ) in November 2011. The searches were restricted to actively published and indexed periodicals. Dermatology journal names were obtained by means of "search by scientific discipline" option in IC,[1] "view subject category" section in SCI [2] and SCI-E,[3] "broad subject terms for indexed journals" option in PubMed/MEDLINE,[4] and "browse by subject" feature in DOAJ.[5] The journal names extracted from index searches were investigated and inappropriate ones from other disciplines were excluded. Some of the journals overlapping in the search were also eliminated. The web pages of all the periodicals in the current journal list were investigated and the data yielded by search through databases was checked.

Origin country and publication frequency were noted for each journal. The last volume number of a journal was recorded as the age of the periodical. Statistical analysis was performed by using SPSS software package (version 16.0, SPSS Inc., Chicago, IL.). Descriptive analyses and sample-T test were used for frequencies and comparisons.

Results

Ninety seven dermatology journals were identified from 28 countries. Pubmed was the largest database with 67 dermatology periodicals. 53 journals were indexed in SCI-E, 26 included in SCI, 25 in DOAJ and in 23 in IC. There were 32 journals published in USA, 11 in UK and 8 in Poland (Table 1). The earliest start year of the dermatology journals currently published in the world was 1893. The mean age of journals was 30.21 ± 3.32 years (range 1–223) (Table 1).

There were 41 actively indexed dermatology journals in 12 European countries. The oldest European dermatology periodical was *Dermatology* with 223 years, from Switzerland and it was also the oldest one of the world.[6] Switzerland had 4 publications in the list and its mean journal age was statistically older than those of other countries of the world although it had even a three-year-old journal (73.50 ± 50.50, $p<0.05$). In PubMed/MEDLINE all dermatology journals published in the UK were referenced. The oldest journal from the UK was *Leprosy Review* with 82 years published quarterly and indexed in SCI, SCI-E and PubMed.[7] Interestingly, although *Actas Dermo-Sifiliográficas* was a

Table 1. Countries in descending order of the number of dermatology journals actively indexed and the ages of the periodicals in the countries

Country	SCI	SCI-E	Pubmed	Index Copernicus	DOAJ	Total	Age (Mean ± SD / Min-Max)
USA	13	24	26	1	5	32	29.97 ± 4.78 / 1 – 131
UK	3	7	11	1	2	11	30.27 ± 7.09 / 4 – 82
Poland	None	None	None	7	2	8	17.88 ± 11.53 / 2 – 98
Switzerland	2	2	3	None	1	4	73.50 ± 50.50 / 3 – 223
Egypt	None	None	None	1	4	4	4.25 ± 1.89 / 1 – 8
Denmark	3	3	3	None	None	3	34 ± 15.52 / 17 – 65
Germany	2	3	3	1	None	3	41.33 ± 16.29 / 9 – 61
India	None	1	3	2	2	3	31.67 ± 15.68 / 3 – 57
Canada	None	2	3	None	None	3	15.67 ± 3.75 / 9 - 22
Turkey	None	1	None	3	1	3	23.33 ± 11.66 / 5 – 45
Japan	1	2	2	None	None	2	45.5 ± 18.5 / 27 – 64
France	1	2	2	1	None	2	27.5 ± 6.5 / 21 – 34
Brazil	None	1	1	1	2	2	26.5 ± 23.5 / 3 – 50
Italy	None	1	1	1	1	2	17 ± 14 / 3 – 31
Iran	None	None	None	2	1	2	8.5 ± 6.5 / 2 – 15
China	None	1	None	1	None	2	14 ± 5 / 9 – 19
Sweden	1	1	1	1	1	1	91
EU	None	1	1	None	None	1	25
Australia	None	1	1	None	None	1	44
South Korea	None	1	1	None	None	1	23
Crotia	None	1	1	None	None	1	19
Argentina	None	None	1	None	1	1	92
Taiwan	None	1	None	None	None	1	29
Spain	None	None	1	None	None	1	102
Slovenia	None	None	1	None	None	1	20
New Zealand	None	None	None	None	1	1	4
Peru	None	None	None	None	1	1	19
Total	26	53	67	23	25	97	30.21 ± 3.32 / 1 – 223

102-year-old journal from Spain it was only referenced in PubMed.[8] *Przegląd Dermatologiczny* had the earliest publication start year in Poland. It was founded in 1906 but only indexed in DOAJ and IC.[9] Turkey had three dermatology periodicals and none of them were included in PubMed/MEDLINE database. *TURKDERM - Archives of The Turkish Dermatology and Venerology* was the oldest one, open access and only SCI-E indexed. France had two periodicals, both were Pubmed and SCI-E indexed.[10] *European Journal of Dermatology* was published in France and referenced in all databases besides DOAJ.[11] *Journal of the European Academy of Dermatology and Venereology* could not be located into only one European country and evaluated it as a periodical of European Union. It was indexed in Pubmed and SCI-E and started in 1991.[12]

Journal of Investigational Dermatology was the oldest journal in the North and South America with 131 years of publication history.[13] South America countries had only 4 journals in the list and the oldest one was *Revista Argentina de Dermatología* with 92 years from Argentina.[14] Fifty percent of dermatology journals indexed in SCI was from the USA (Table 1).

Among 11 journals published in Asia the oldest one was *Journal of Dermatological Science* from Japan indexed in Pubmed, SCI and SCI-E.[15] This journal was the only periodical from Asia indexed in SCI. India had three journals in the list and all of them were indexed in PubMed (Table 1). *Indian Journal of Dermatology, Venereology and Leprology* was open access and referenced by four databases, PubMed, SCI-E, DOAJ and IC.[16]

Egypt was the only country with four journals from the continent of Africa. All Egyptian journals were younger than five years, open access and indexed in DOAJ.

Most journals (36 %) were published bimonthly. The mean age of the monthly and bimonthly publications were 48.11 and 33.97 years respectively and there was no statistically significant difference. 14 (14.43 %) journals had irregular publication and most of them (92.85 %) were open access. *Acta Dermato-Venereologica* (91 years, Sweden) was the unique open access dermatology journal indexed in SCI and therefore it could be referenced by all 5 databases.[17] DOAJ had the newest journals that included even one-year-old ones.

Discussion

There are 196 *countries* in the *world* although some controversy still exists. Only 28 countries had actively indexed dermatology journals in the international databases investigated in this study. Most journals were from developed or developing countries. Whether they are indexed in well-known and popular international databases is one of the major factors that affect journals' academic influence in the professional field in the world. *The current study is the first report to evaluate the dermatology journals actively published and indexed around the world.*

"Globalization" is a notion most popular now than ever before but we could not talk about a globalized world of dermatological literature. For instance, although China is the world's most populous country with 1.3 billion people it had just two dermatology journals indexed in international databases. *Hong Kong Journal of Dermatology and Venereology* was the only Chinese dermatology journal indexed in SCI-E.[18] There was no journal from China referenced by Pubmed or SCI.

Vlassov and Danishevskiy reported that there were only 30 Russian periodicals referenced in PubMed/MEDLINE and none of them was a dermatology journal.[19] Although the first independent Russian dermatology periodical was founded in 1901, no dermatology journal from Russia and former Soviet Union States indexed in databases was found.[20] The cause of this situation might be a result of the political and medical isolation of the "iron curtain" countries. Poland was the only former "iron curtain" country which had indexed dermatology periodicals in the journal list.

Siegfried et al. reported that African dermatology journals were poorly represented in the international databases.[21] The results of this study confirmed this opinion. Only 4 Egyptian periodicals were indexed by databases searched. Two of them were published irregularly and the oldest one (*Journal of the Egyptian Women's Dermatologic Society*) was eight years old.[22] Regularity of publication and accessibility are important criteria for journal selection in the international databases and African journals will have to improve themselves if they are to increase the likelihood of being indexed in databases.

It is noteworthy that one limitation of this study is that although the medical databases used in this study are comprehensive, all international popular databases in the world could not be searched in this study. *There are two reasons for this limitation. First, some medical databases do not provide free access and public search. Secondly, journals are not arranged by subject category in some medical indexes and searching by keywords is not an efficient method. In conclusion, this article can serve as a preliminary study and further studies including national databases are needed.*

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Acute pancreatitis associated with Herpes Zoster Virus infection in an immunocompromised adult

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Abstract

Although a wide variety of infectious agents have been implicated in the etiology of acute pancreatitis since as early as 1817, viral pancreatitis is a rarely encountered entity in the literature. Here we report a patient, who used adrenocorticotropic hormone and immunosuppressive drug intermittently for two years, developed acute pancreatitis in association with herpes zoster virus infection. The abdominal computed tomography scan helped to diagnose acute pancreatitis and observe the curative effect, as the serum amylase was within normal limits. Herpes zoster virus infection should be included in the conditions causing acute pancreatitis, when common noninfectious factors have been excluded in this case. Although varicella infection had been associated with acute pancreatitis in children previously, viral pancreatitis because of herpes zoster virus in adult had not been reported previously. The results reported here will help to improve our attention to the diagnosis of atypical pancreatitis in those patients with abdominal pain and virus infection such as herpes zoster virus.

Key words: Acute pancreatitis, Herpes Zoster Virus, immunocompromised.

Introduction

The appropriate diagnosis and treatment is very important for acute pancreatitis. Otherwise it will defer to acute severe pancreatitis, which has high mortality rate. We generally exclude pancreatitis in those patients who complain abdominal pain by examining amylase in the blood or urine. Especially in those patients with high common risks, such as cholelithiasis, binge eating or excessive drinking, the bellyache symptoms are usually typical and serious, the diagnosis is easy to make according to the elevated blood or urine amylase. However, acute pancreatitis might also occur in other rare conditions, such as hypercalcemia, hypertriglyceridemia, drug allergy and poisoning and various infections. Pancreatitis induced by such causes is often mild, and the timely diagnosis and treatment may have crucial clinical significance.

Although infectious agents constitute a small percentage of the conditions that predispose to acute pancreatitis, a wide variety of infectious agents has been associated with acute pancreatitis [1]. They include bacteria (Mycoplasma, Legionella, Leptospira, Salmonella), fungi (Aspergillus), parasites (Toxoplasma, Cryptosporidium, Ascaris) and viruses (mumps, coxsackie, hepatitis A, B, C&E, cytomegalovirus, varicella-zoster virus, herpes simplex virus, human immunodeficiency virus, Epstein–Barr virus, rubella, adenoviruses, influenza A&B, measles virus, rotavirus, dengue virus) [1-10]. Although varicella infection had been reported to be associated with acute pancreatitis in children previously [11-13], herpes zoster virus infection was a rare cause of acute pancreatitis in adult. Here we reported an immunocompromised adult who suffered from acute pancreatitis which might be due to herpes zoster virus.

Case report

History of present illness

A 68-year-old man was referred to us because of abdominal pain and distention for eight days. The patient was complaint of left inferior abdominal pain, radiating to the lower back, and abdominal distention. There were batches of herpes at the left inferior quadrant of the abdomen and the left groin region. No other symptoms such as vomiting, chest tightness, diarrhea, cough, runny nose and headache were evident.

A 68-year-old man was referred to us because of herpes and abdominal pain for eight days. Batches of herpes at the left inferior quadrant of the abdomen and the left groin region occurred eight days before. The patient was complaint of left inferior abdominal pain, which was sharp and paroxysmal at very beginning. The pain gradually turned persistent, radiating to the lower back, and accompanying abdominal distention. He also had a low fever around 37.5-38.0ºC in the recent three days. No other symptoms such as vomiting, chest tightness, diarrhea, cough, runny nose and headache were evident.

History of past illness

The patients had a history of hypertension for more than 30 years, and Procardin, Clonidine were used for anti-hypertensive therapy. He was also given the renal biopsy 2 years before, and the pathological finding was membranous nephropathy (stage I-II) with glomerular sclerosis, which was consistent with hypertensive renal damage. Prednisone (10mg BID) and Tarcolimus (25mg BID) were used for his membranous nephropathy. Half a year later, the patient stopped using Prednisone and Tarcolimus by himself because of gastrointestinal bleeding. Prednisone (10mg BID) and Tripterygium wilfordii (20mg BID) were given again two months ago due to the increased urinary protein and aggravated renal function. The patient did not report any similar symptoms in the past, had no history of tumor, drug allergy, trauma and genetic diseases, and denied any alcohol or smoke consumption.

Physical examination

On admission, his vital signs were as follows: heart rate 102/min, blood pressure 155/88 mmHg, oral temperature 37.8°C, and respiratory rate 18/min. Pulse oximetry showed a normal O2 saturation of 98%. No enlarged lymph nodes were found in the neck and supraclavicular fossa. Skin and sclera were not icteric, and mild anemia appeared. Heart and chest auscultation did not reveal any abnormal findings. Physical examination revealed abdominal prominence and mild tenderness at the left inferior abdomen. No rebound tenderness and muscle tension were evident. Murphy sign was negative. Liver and spleen were not palpable below the costal margin. Batches of herpes were found at the left inferior quadrant of the abdomen and the left groin region. Bowel sounds was normal (4/min). Lower extremities were not edema. Negative neurological examination revealed no abnormalities.

Laboratory findings

Inflammatory and infective index did not suggest an acute inflammatory or infective condition. WBC was 9300/μL (neutrophils: 7500/μL, lymphocytes: 1000/μL). The normal count of lymphocytes seemed incompatible with simple viral infection, which might be due to his long-term use of the steroidal and immunosuppressive drugs. Hemoglobin was 87g/L, platelet was 16900/μL, C-reactive protein was 20.20mg/L, and erythrocyte sedimentation rate was 70mm/h. No bacteria were found in blood cultures for seven days. Autoantibodies were all negative. Clinical biochemistry showed: serum amylase (ranged from 64 to 171 U/L) and urine amylase (ranged from 30 to 80 U/L) were within normal range. The other pancreatic enzyme levels, such as trypsin and lipase could not be measured in our hospital. Liver function, fasting glucose, coagulation tests and plasma electrolytes including calcium were also normal. His renal function was impaired: creatinine was 250umol/L, and urea nitrogen was 18.71mmol/L. Albumin was decreased (26.4g/L), which was due to the increased urinary protein (24-hour urinary protein was 12.14g). The hyperlipidemic profile was also seen: triglyceride was 2.23mmol/L, total cholesterol was 7.50mmol/L and LDL cholesterol was 4.55mmol/L. Carbohydrate antigen-199 was slightly increased (41.3U/mL). Occult blood test was negative.

Abdominal ultrasound as well as computed tomography scan revealed no stones or sludge either in the gallbladder or in the common bile duct, but gallbladder wall thickening with surrounding edema and exudation could be seen. A computed tomography scan was also performed after intravenous contrast administration and additionally revealed diffuse edema of the pancreas with a lot of surrounding exudation (Figure 1). Upright abdominal plain film showed intestinal gas accumulation in the abdomen and multiple small fluid levels.

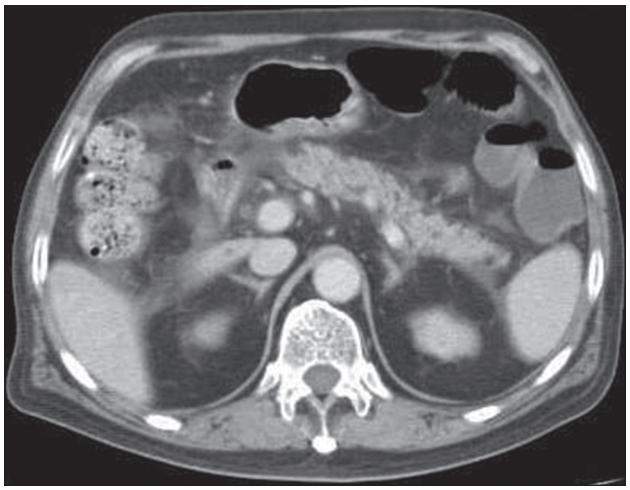

Figure 1A. The diffuse edema of pancreas with exudation surrounding the neck and tail, the thickening of the left prerenal fascia

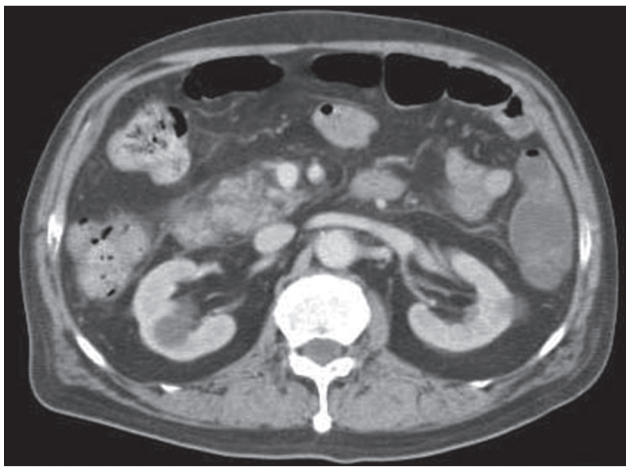

Figure 1B. Exudation surrounding the head of the pancreas

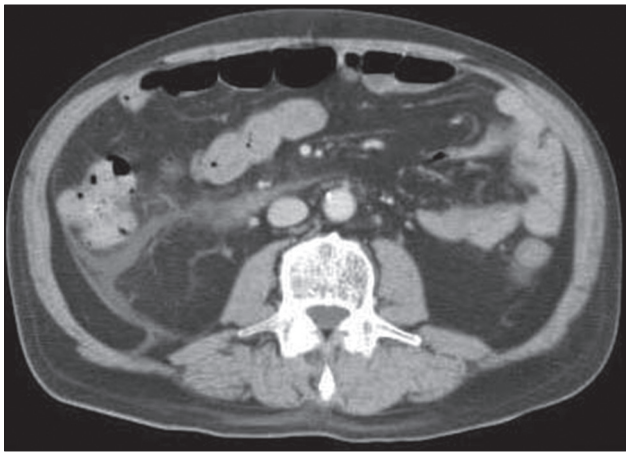

Figure 1C. Hydrops in right paracolic sulci

Figure 1. A computed tomography scan reveals acute pancreatitis in an immunocompromised 68-year-old man under herpes zoster virus infection (Before therapy)

Diagnosis and treatment

Laparozoster was diagnosed by an experienced dermatologist according to the zonal distribution of herpes on the left side of the inferior abdomen, accompanying paroxysmal neuralgia. The abdominal pain of the patient was also confined to the left inferior abdomen, and it occurred with the herpes simultaneously, so we considered that he simply suffered from the laparozoster at the very beginning. However, the patients also complained of abdominal distention soon later, and the abdominal discomfort radiated to the lower back. Further examination by computed tomography scan revealed a lot of surrounding exudation of the pancreas. Acute pancreatitis was then diagnosed although the serum and urine amylase remained normal all the while. Upright abdominal plain film also implied transient intestinal paralysis, which supposed to be induced by pancreatitis.

Since admission, the patient remained fasted and was given intravenous nutritional support. Octreotide was subcutaneously injected to reduce the secretion of pancreatin, Omeprazole was intravenously injected to inhibit gastric acid for improving gastrointestinal symptoms, Cefoperazone and Metronidazole were preventively used for anti-bacterial therapy, and Famciclovir tablets were given for anti-virus treatment. Other therapy included local wet compress with Rivanol, intramuscular injection of Mecobalamin for neurotrophic purpose, and analgesics by Tramadol Hydrochloride and Neurotropin. Epidural injection with lidocaine hydrochloride and Diprospan through intervertebral space between Lumbar 1 and 2 were also performed to reduce the pain. His upright abdominal plain film was turned into normal, and the abdominal distention disappeared 2 days later. Surrounding exudation of the pancreas was also much less one week later. Feeding was then continued with soft and gradually solid food. The patient was discharged 9 days after admission without digestive symptoms and signs, although he was still bothered by post-herpetic neuralgia. The pancreas and the surrounding interspaces recovered normal two weeks later (Figure 2).

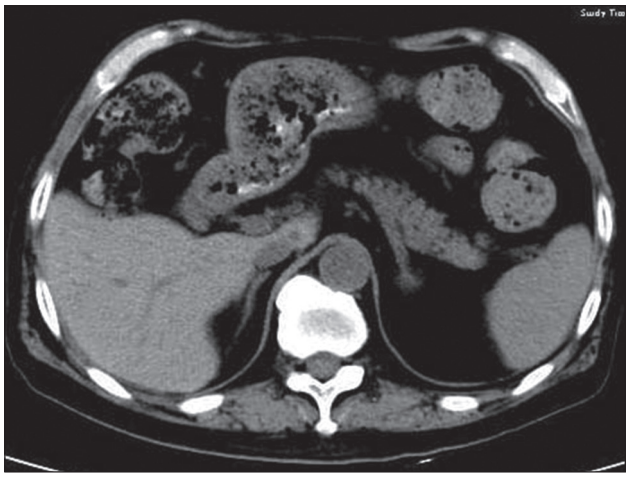

Figure 2A. The exudation surrounding the pancreas disappeared, and the prerenal fascia recovered to normal

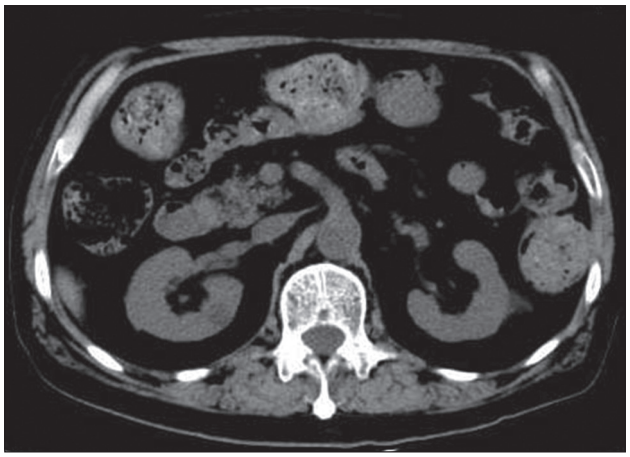

Figure 2B. Exudation surrounding the head of the pancreas disappeared, and the interspaces of the bilateral prerenal fascia recovered to normal

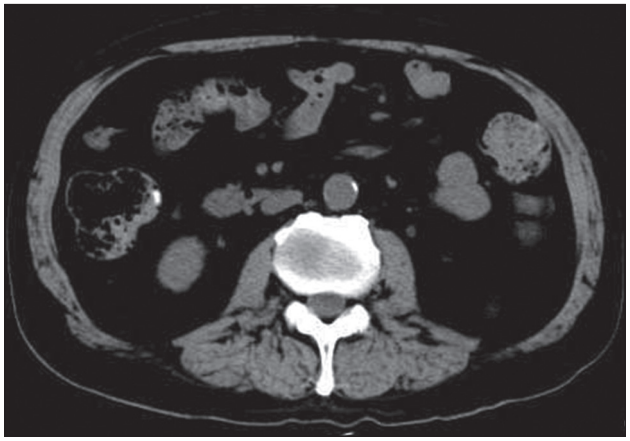

Figure 2C. Hydrops in right paracolic sulci disappeared

Figure 2. A computed tomography scan reveals normal pancreas in an immunocompromised 68-year-old man under herpes zoster virus infection (After therapy)

Discussion

The major predisposing conditions for acute pancreatitis include biliary obstruction such as gallstones, overeating and excessive drinking in China as well as in the Western countries [14]. Hyperlipidemia, hypercalcemia, acute thrombosis of pancreatic small vessels, surgery or trauma, drugs or some genetics disorders might also lead to this disease [14]. However, the aforementioned causes did not account for this case. About 15-25% of pancreatitis episodes are of unknown origin [14], some of them might be attributed to kinds of infection [1]. The virus or bacteria might enter the pancreas though the circulation of blood and lymph. Recently, quite a few case reports are about hepatitis viruses associated with acute pancreatitis. Viral acute pancreatitis because of varicella infection is relatively rare, and it only found in immunocompetent children [11-13]. No pancreatitis has been reported in adults previously.

Acute pancreatitis of this case was considered to be caused by herpes zoster virus infection with no other common causes of the disease was evident. Visceral dissemination of herpes zoster may follow cutaneous or even gastric dissemination in immunocompromised patients [15]. Primary infection with varicella zoster is usually featured by a generalized vesicular rash without significant systemic complications. However, encephalitis, pneumonitis, nephritis, pancreatitis, Reye and Guillan-Barre syndrome transvers myelitis, myocarditis and even multiorgan failure have also been reported previously [16]. Generally, pancreatic injury is mild in 80% of patients of acute pancreatitis, and the remaining patients have a severe disease with local and systemic complications [14]. Similar with the most previous reports about viral pancreatitis, our case also presented simply edema pancreatitis, and the clinical manifestations were atypical. The abdominal pain was limited within the left inferior abdomen, with abdominal distention and the discomfort of the lower back. No systemic inflammation or elevated amylase was evident. Even the serum and urine amylase remained normal during the course of the disease. It was a limitation that the other pancreatic enzyme levels, such as trypsin and lipase could not be measured in our hospital. A prompt diagnosis owed to the findings of computed tomography scan.

Currently, it is not yet clear whether infectious agents can cause pancreatitis alone. Here we reported a viral pancreatitis associated with herpes zoster virus infection in an immunocompromised patient with membranous nephropathy, who was using adrenocorticotropic hormone (Prednisone) and immunosuppressive drug (Tripterygium wilfordii). It had been reported that glucocorticoid could induce pancreatitis in children [17], because the hormones might prompt pancreatic secretion and increase pancreatic juice viscosity. The long-term use of Prednisone could also lead our case to a relative adrenal insufficiency under stress, which might trigger the development of acute pancreatitis [18-19].

In summary, although rare, viral agents including herpes zoster virus should be considered in the differential diagnosis of acute pancreatitis in those immunocompromised adults with abdomen pain, especially when no other common causes of the disease is evident.

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Scientific and educational aspects of the structures of amino acids

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Abstract

This paper is based on research needed to show that the final transfer of knowledge, such as usual class lectures, nowadays, gives poorer results than other, more modern forms of education. Selected teaching topics are presented in the usual way in the control groups and the innovative way, taking into account the scientific facts that are not represented in the academic literature, in the experimental groups. Given that modern education seeks a functional knowledge of students, knowledge that can be applied, this research proves the point. Students are much more motivated to work, to participate actively in the learning process, if they know that they are expected connect to previous knowledge, reasoning, research, or other forms of engagement in the classroom. During the so-called ex cathedra lectures, their motivation is much smaller, minority students are involved in the work, do not ask why, how, what, but a priori accept information. The results of knowledge tests, analyzed by descriptive statistics clearly show better achievements in the experimental groups.

Key words: functional knowledge, education, teaching methods, curricula.

Introduction

Amino acids are essential and invaluable for live systems, so it is imperative that high school students, especially high school and school of biomedical orientation, fully master their structural characteristics, as a prerequisite to a fuller comprehension and understanding of their functions and roles, primarily within the protein, but also independently of them, as individual molecules. It includes functional, applicable, knowledge, which unfortunately, is not present in sufficient degree, and which speaks in favor of the results obtained in international tests of students conducted in the last few years (PISA). Hence arises the actuality of this topic, especially in natural sciences, where the results are weaker on average. Formation of applicable knowledge requires changing the usual way of executing instruction (transfer of ready knowledge) to learn through logical connections, reasoning, performing simple research, practical work, etc. Already, amino acids and proteins are for decades, as the program unit, located in the curricula of chemistry, biochemistry and biology of most high schools, when we talk about three or four year program schools, with more or less teaching hours of chemistry, both in our country and the world in general.

The aim of this work

Review of existing ways of interpreting the structure of amino acids, shows the possibility of the presence of many other, different approaches to teaching this subject. Taking into account the age of the material which it is intended for, it makes sense to assume that the currents approaches to the problem of studying the structure of amino acids is inadequate. In most textbooks, both high school and college, the structure of amino acids is given in tabular form often inadequate classification, almost without any explanation of why such and such 20 amino acid protein is building and overall living world and without explaining why such a classification, and not some other and different.

What, however, is the most annoying, looking at the methodological and methodological point of view, is an inefficiency in the presentation, and therefore in the acquisition of knowledge by students. The consequence is a well known fact that students, even students rarely know how to list all 20 protein amino acids, not to mention their lack of knowledge of their structure (with the knowledge

to write the structural formula). A detailed analysis of the scientific and professional literature during the study (Carson 1982, Stryer 1991, Pine 1994, Koracevic 1996, Tic 1997), leads to a conclusion that the teaching of a single approach does not exist. To overcome this, we have made an attempt to build a possible unified approach to the study of the structure of amino acids and their function.

Methods of work

The survey was planned to be didactic-methodological experiment carried out in two schools. The idea was that it should be carried out in a vocational school, and one high school. Thus, the survey covers three fourth grade classes of medical school, "Dr. Milenko Hadzic" in Nis and one class II Grade School "Svetozar Marovic" in Nis (in these classes amino acids are taught within biochemistry curriculum in Medical School and chemistry in High School).

Before the research started school psychologists had tested the intelligence of students and came to the conclusion that there was not significant difference between the groups. In all three departments of the Medical School the same teacher taught biochemistry throughout the school year, as it is in the same High School chemistry teacher taught the entire school year. The decision about which classes will be control and which the experimental groups was made by the method of random selection (one class of medical school department and gymnasium were in control group K_1 and K_2, while two classes of medical school is an experiment in group E_1 and E_2), which means the study included 136 students: 65 in the control group and 71 in the experimental. The nature of research required carrying out research in conditions of normal school situation, without equalization of the groups, provided that the classes have about the same pick overall success and about the same intelligence.

The goal was to provide a completely natural and appropriate occasion, to avoid making any changes in the usual working atmosphere and to eliminate the presence of the experimental situation. This requirement was insisted on at the cost of failure to eliminate a variety of other disorders, which inevitably led to inconsistency of experimental conditions. Because of this even common methodological errors that occur in these situations could not have been avoided (Kundacina, Bandur, 2000):

Errors "S" types, which are related to the subjects participating in the experiment could not be avoided, due to the fact that the research did not work on the standardization group according to their prior knowledge and intellectual capabilities, but are taken as subjects were in the experimental department or the control group. Despite the intelligence test showed no statistically significant differences between the groups, errors of this type to some extent in this situation may appear.

Errors "G" types, perhaps, could come to the fore. These are the errors that stem from differences between the groups with regard to the differences in the classes of teachers teaching aids and school equipment, and differences the premises and didactic-methodical experiments and the like. This difference may be the most prominent among the groups that come from two different schools. However, there were no statistically significant differences between the groups in the same school, because we tried to provide the same work conditions for both control and experimental groups.

Errors "R" types could appear stemming from differences caused by repeating the experiment. In repeating the experiment in the other school was carried out under different conditions, in different place and different time.

Regardless of the possible impact of these methodological errors, the fact that students in the experimental and control groups are usually randomly selected, gives us the right to assume the masking effect, or even the elimination of many of these errors. Also, constant and careful control of the experimental situation, strictly monitoring the changes that occur under the influence of experimental factors that caused the mentioned methodological errors do not significantly come to the fore.

The basic prerequisite to obtain the relevant indicators of the impact of a new curriculum content, teaching material of amino acids, was to give a lecture in the control and experimental groups, and then to test knowledge and perform a complete statistical analysis of the quantitative and qualitative analysis of the results.

In Medical School teaching selected units were realized the same day in the three departments

which had biochemistry class. The control group listened to the teaching content of the amino acid structure treated in the manner provided in the curriculum, and the experimental group listened to the same instructional content in processed innovative way (Rakocevic 1996, Dlyasin 1998). Both lessons in control and in the experimental classes were completed by the same subject teacher. At the end of the class period of 15 minutes in the control and experimental group students solved problems given in the knowledge test (test output). Preceding this test the students were given general knowledge test in chemistry (entry test). In high school, the same procedure was repeated, with emphasis, that there is content processed as required by the curriculum (control group).

Research techniques, as well as specific procedures in specific phases of research, will be used as follows:

1) At the stage of data collection:
   a) Tests
   b) Method of observation
2) At the stage of data processing:
   a) Development of statistical tables and descriptions
   b) Statistical computing and reasoning

Within these research techniques the following research instruments will be used:

1) Intelligence tests (the results of the school psychologist)
2) Knowledge Tests

In an effort to determine the extent to which students who are the objects of our study, gained knowledge we adopted an approach to the material, whenever possible, by direct examination of the students themselves. Direct examination of educational research is usually done by testing.

To measure the results achieved by the respondents we constructed properly proficiency tests. When deciding on the type and kind of knowledge tests we started from the very nature of research and the types of criteria used in tests that best suit their purpose and make it possible to detect the difference (if any) between the easiest and the most difficult tasks (Nesic 1996).

Between the two types of tests: essay-type tests and short answers, the choice was purely for the latter, because they allow a more objective judgment about achievement, and easy evaluation whether the task is solved completely, or in part (1/2 or 1/4), if the questions are well formulated.

The first test, entrance test, intended for checking general knowledge of chemistry, was made pursuant to a program for first and second grade secondary school (gymnasium socio-linguistic orientation and vocational schools). This test made it possible to test all the students. The second test, the test output is intended for measuring the success of students who achieve mastery in a specific curriculum subjects, material on the structure of the protein amino acids.

Conditions of research decided on the sample size of statistical methods to analyze the results. First, using conventional descriptive statistics, the results were prepared for analysis. For each group of subjects, and for each parameter, clearly, in the form of tables, are given calculated basic statistics: mean (mean), 95% confidence interval for the mean value of occurrence in the population from which the sample was taken (confid.95%) , standard deviation (SD) and variance (Varian), the error standard deviation (SD err.), data on the mi-

Table 1. Descriptive statistics on the results of the entrance test

Group	No. of st.	Mean	Confid. 95%		Sum	Min	Max	Range	Varian.	SD	Err. SD
K_1	37	5.89	5.54	6.25	218.10	4.00	7.75	3.75	1.13	1.06	0.18
K_2	28	5.10	4.61	5.58	142.66	2.17	8.33	6.16	1.60	1.26	0.24
E_1	38	4.01	3.80	4.22	152.34	2.83	5.33	2.50	0.41	0.64	0.10
E_2	33	5.01	4.63	5.38	165.18	3.00	6.25	3.25	1.09	1.05	0.18

Table 2. Descriptive statistics on the output test results

Group	No. of st.	Mean	Confid. 95%		Sum	Min	Max	Range	Varian.	SD	Err. SD
K_1	37	7.23	6.70	7.76	267.50	3.00	9.25	6.25	2.52	1.59	0.26
K_2	28	7.10	6.50	7.69	198.75	4.00	9.00	5.00	2.35	1.53	0.29
E_1	38	8.79	8.51	9.07	334.00	7.50	10.00	2.50	0.72	0.85	0.14
E_2	33	8.88	8.51	9.25	293.00	6.00	10.00	4.00	1.11	1.05	0.18

Table 3. Chi-square change from complete success

Achievement	Group	Hi-calculated	Hi-Line	Probability
A	K_1 (37)	14.20	71.30	0.999590
	K_2 (28)	8.70	59.40	0.999680
	E_1 (38)	10.40	87.90	0.999995
	E_2 (33)	7.00	85.50	0.999999
B	K_1 (37)	66.43	15.91	0.001506
	K_2 (28)	71.68	5.96	0.000007
	E_1 (38)	135.73	8.86	0.000001
	E_2 (33)	85.82	7.00	0.000001
C	K_1 (37)	37.45	33.30	0.402503
	K_2 (28)	29.92	23.05	0.317921
	E_1 (38)	8.25	76.40	0.999999
	E_2 (33)	7.70	70.60	0.999999

nimum (min) and maximum (max) values in a set of basic features (range) and the range of values that they cover (sum).

Results

To better understand the kind of results we have arrived at this research, it is, of course, necessary to make their quantitative and qualitative analysis. After teaching in the departments where the groups differed only in introduced the experimental factor in the experimental group, a new approach to teaching the structure of the protein amino acids, we measured the results.

The basic statistics given in Tables 1 and 2, with Table 1 refers to the entry test, and Table 2 to the test output.

Table 3 shows calculated chi square for the distribution of data on the success of each group separately and theoretical distribution in relation to the results of the entrance test with 50% achievement in Section A, 100% achievement in Section B and the output of the test with 100% achievement in Section C.

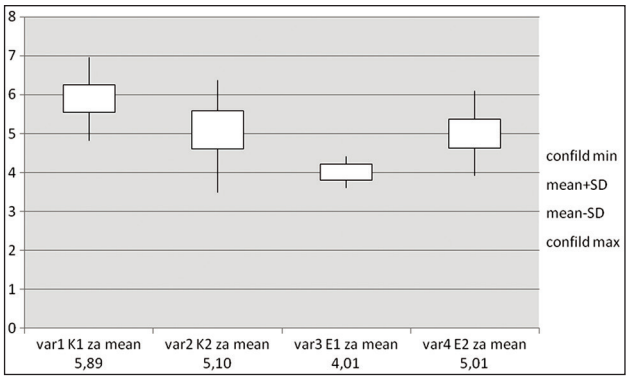

Figure 1. The mean value of success in the entrance test

Table 4. Percentage of answers to the questions of the entry test

Question	K_1 (%)	K_2 (%)	E_1 (%)	E_2 (%)	Question	K_1 (%)	K_2 (%)	E_1 (%)	E_2 (%)
1.	27.02	35.71	24.24	0.00	17.	9864	60.71	90.90	65.78
2.	37.83	39.28	15.15	0.00	18.	2.70	10.71	16.66	1.31
3.	86.48	100.00	93.93	100.00	19.	60.81	30.35	54.54	63.15
4.	94.59	35.71	84.84	34.21	20.	1.13	0.00	3.03	0.00
5.	94.59	92.85	60.60	27.63	21.	0.00	25.00	57.57	42.10
6.	72.97	58.92	62.87	67.76	22.	55.40	73.21	0.00	0.00
7.	64.86	48.21	13.63	10.52	23.	78.37	96.42	84.84	98.68
8.	32.43	75.00	60.60	27.63	24.	75.67	82.14	69.69	98.68
9,	72.97	59.82	62.87	67.76	25.	59.45	67.85	87.87	89.47
10.	33.78	12.50	0.00	0.00	26.	74.32	66.07	6.06	2.63
11.	40.54	21.42	0.00	0.00	27.	89.18	78.57	93.93	81.57
12.	78.37	73.21	89.39	94.73	28.	89.18	21.42	87.87	57.89
13.	0.00	4.46	0.00	0.00	29a.	70.27	76.78	68.18	50.00
14.	40.54	8.92	13.63	0.00	29b.	70.27	76.78	68.18	52.63
15.	87.83	96.42	42.42	43.42	30.	27.02	10.71	12.12	1.31
16.	100.00	64.28	90.90	65.78					

Table 5. Percentage of answers to the questions of the test output

Question	K_1 (%)	K_2 (%)	E_1 (%)	E_2 (%)	Question	K_1 (%)	K_2 (%)	E_1 (%)	E_2 (%)
1.	94.44	100.00	100.00	100.00	6.	84.02	68.75	98.48	96.05
2a.	79.16	89.28	93.93	98.78	7.	76.38	98.21	96.96	93.42
2b.	79.16	89.28	93.93	98.48	8.	45.83	46.42	62.12	44.73
3a.	66.66	37.50	81.81	100.00	9.	83.33	85.71	100.00	97.36
3b.	66.66	37.50	81.81	100.00	10a.	11.11	0.00	100.00	97.36
3c.	68.05	37.50	81.81	100.00	10b.	5.55	0.00	100.00	97.36
3d.	68.05	37.50	81.81	100.00	10c.	11.11	0.00	93.93	97.36
4.	81.25	67.85	100.00	98.48	10d.	11.11	0.00	93.93	97.36
5.	20.83	57.14	59.09	63.15	10e.	5.55	0.00	93.93	97.36

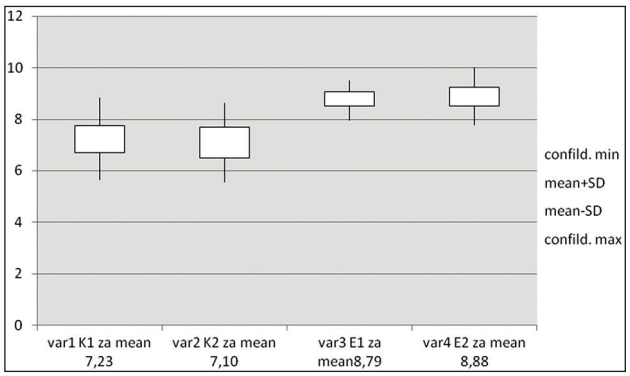

Figure 2. The mean value of success on the exit test

Figures 1 and 2 show the mean (arithmetic mean) and the deviation from the mean value of the entry and output test. In Tables 4 and 5 can be traced the results achieved with each and every issue of the input and output of the test expressed as a percentage.

Discussion

As previously mentioned, the analysis of the results must be quantitative and qualitative. For a quantitative analysis of the results are shown in Tables 1, 2 and 3, and Figures 1 and 2, while qualitative

analysis supported by tables 4 and 5 Tables 1 and 2 show the results obtained in the entrance and exit test done using conventional descriptive statistics.

Measured mean values, achieved success by groups (K_1 and K_2 control group; E_1 and E_2 experimental group) on the entrance test, it is a test of prior knowledge, it goes in this order: K_1 (5.9), K_2 (5.1), E_1 (5.0) and E_2 (4.0). This means that all four groups achieved approximately the same success and it is at the level of 50% achievement. On the other hand, it also means that, from the standpoint of prior knowledge of the relevant material chemistry between the groups there was no significant difference.

That this is so is slowed by calculated the chi square for the distribution of data on the success of each group, and the theoretical distribution, the entrance test, in which each student had exactly 50% achievement (Table 3, section A). The table shows that, the calculated value of chi square is very low compared to the theoretical limits, which means that there are no statistically significant differences, primarily in relation to the theoretical frequency, and consequently between the groups.

These results are obtained when we observe the 50% achievement. However, if we look at the success of students in the entrance test of knowledge in relation to the maximum possible (100%) achievement (Table 3, Section B), the calculated chi-square shows that there is a statistically significant difference between each group and the theoretical frequency in which achievement of every student suited the obtained maximum number of points (which is, otherwise, the final requirement in criterion achievement tests).

From the point of view of the same methodological procedure it makes sense to compare the success of the control and experimental group achieved the exit test, in which the impact of the introduced experimental factors is achieved. Sequence groups (Table 2) according to their performance is as follows: K_1(7.1), K_2 (7.2), E_1 (8.8) and E_2 (8.9). As in section B of Table 3, and here, in section C of the same tables chi square is calculated in relation to the theoretical distribution, which represents 100% achievement. We see that only the success of the experimental group ($E_1$and E_2) is approaching that distribution and there it is not statistically significant differences. In the control group (K_1 and K_2), however, in the exit test there was a statistically significant difference (chi-square calculated value greater than the limit value, with a corresponding probability).

Based on the results shown in these tables it is evident that the advantages of the entrance test were control groups, while the output test case for the experimental groups, as shown in Figures 1 and 2 show that the mean results of the control and experimental groups, with deviations of the mean.

In this study, due to the specific effects of the experimental factors it is not possible to separate the qualitative analysis from quantitative. Nevertheless, it is necessary to analyze the students' responses to individual questions of cognitive tests and the results compared with the results of quantitative analysis. From Table 4 and 5 it can be seen that the E_1 and K_1 groups respond differently to certain questions, although these differences are not large. At the entrance test K_1 group students gave better answers to 22 questions, and the E_1 group students better answer 9 questions. If we take into account the questions properly answered by 50% of the students, then the ratio of 19:18 is in favor of K_1 group. After the lecture and after the introduction of the experimental factors on the exit test, the ratio is significantly altered: E_1 group of students better answered most of the questions in relation to students K_1 group. Also, the students group E_1 correctly answered to 16 out of 18 questions in 80% of cases, while students K_1 group achieved this result only in the answers to 7 questions.

In group E_2, and K_2 results before and after the introduction of the experimental factors are also given in Tables 4 and 5. At the entrance test students K_2 group better responded to 19 questions, and the students in group E_2 to 11 questions. Questions properly answered by over 50% of students are in relation to 18:16 in favor of K_2group. After the lecture and after the introduction of the experimental factors on the exit test, the ratio is as in the first case greatly altered: E_2 group of students responded better on almost all issues pertaining to students K_2 group. From the point of 80% of the achievements of this ratio is 16:5 in favor of the E_2 group.

Comparing results shows that K_1 and K_2 groups were proportionally better on the general knowledge test compared to E_1 and E_2 group, however, after explaining concepts (introduction

of the experimental factor) in terms of the structure of amino acids arranged in ten molecular pairs, far better results are E_1 and E_2 group to exit test, compared to the K_1 and K_2 group.

After comparing the results of the second test shows that for the majority of students in all groups were relatively easy questions: 1, 2a, 2b, and 7 whose answers contain the most elementary knowledge of the amino acids. For the control group more serious questions were: 3a, 3b, 3c, 3d, 10a, 10b, and they showed scores below 50% (0 to 37.50%) while students E_1 and E_2 group answered 81 - 100% to the same questions.

For all groups of students more difficult question was the issue number 8, although the percentage of students who showed better performance was in experimental group.

As we can see, and on the basis of qualitative analysis, it can be concluded that the experimental group students scored far better in comparison to the control group.

Conclusion

By systematic analysis of presented research results, we can conclude:

- By comparing the results of the control and experimental group achieved the knowledge tests, it was concluded that the basic assumption is set at the beginning of the research, the students will achieve better results if the subject matter of the amino acids is interpreted in terms of their structure distinctions in amino acid pairs in fully checked, especially if a criterion of success of the entire department is taken into account. In all cases, the experimental group did better on achievement tests. Differences in results are statistically significant at the level 0.05 and at the level of 0.01.
- It was confirmed that innovations of teaching provide applicable knowledge better accepted by students (experimental group) compared to the final transfer of knowledge (the control group).
- There is no doubt that the modern way of life, where the information can be quickly and efficiently found requires modern teaching that meets the needs of students who show a tendency to be more active participants, not passive observers in the learning process in schools.
- These and similar findings can be found in studies in any field, which, among other things, can be seen in modern scientific periodicals (Lotfi, Shahvarani, Moradi 2012th, Mehmet Hakan, Tanner 2012th, Nikolic, Radoicic, Nesic 2011th).

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The incidence of venous thromboembolism within patients with digestive malignancy

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Abstract

Background/Aim. There is a strong causal-consequential bond between carcinoma and intrahospital mortality twice or three times. It also contributes to a progress of tumor and indicates to more aggressive type of tumor. The aim of the research was to confirm a frequency of TDV within digestive malignant patients and to evaluate accuracy and reliability of a diagnostic model of an earlier revealance of TDV, especially its asymptomatic forms.

Methods. The evaluation of a degree of a clinical probability of TDV within 296 available patients with different localisations of digestive malignancy, was carried out by using contemporary Well's model. Within 148 patients of the examined group, the earlier detection of TDV was carried out by combining D-dimer and ultrasound diagnosis (D-dimer diagnostic protocol), according to a determined algorhythm evaluated by a degree of the clinical probability of TDV. The detection of TDV within patients of controlled group (148) was carried out by series of ultrasonography. After ten days of follow-up period, the reliability and sensitivity of diagnostic protocols had been found.

Results. The biggest frequency of VTE was marked within patients with cancer of colon (30.3%) and rectum (31%). Furthermore, there are also patients with pancreatic cancer (26.5%) and gastric cancer (17.6%). There were no cases of VTE within patients with liver cancer. Popliteacrural TDV are significantly more frequent (61.1-66.6%) than iliac-femoral TDV localisations (33.3-38.9%). Clinically unrecognised ('mute') TDV are vitally more frequent (71-82%) than symptomatic forms of the illness (18-29%). Only one case of VTE (0.87%) has been registered in the examined group, which is far less significant ($p<0.05$) in comparison to the controlled group, where 11 cases of VTE (8.46%) have been confirmed. The risk of VTE appearance within patients below 65 years is bigger than below mentioned limits – 25 times according to values of D-dimer>230μg/L, 50 times>2000μg/L, and 30 times>4000μg/L. In comparison to a series of ultrasound diagnosis, D-dimer diagnostic protocol indicates a statistically significant higher level of sensitivity, NPV and diagnostic efficieny in the early detection of VTE.

Conclusion. High values of D-dimer, especially those >2000μg/L, show a strong evaluation of VTE within patients with digestive malignancy younger than 65 years. A concerning frequency of VTE within these patients requires a usage of well-known and highly sensitive D-dimer diagnostic protocol, whose aim is the early detection, as well as a regular use of thromboprofilaxy in all cases of detected colorectal, pancreatic and gastric cancer.

Key words: Digestive malignancy, gastric cancer, colorectal cancer, pancreatic cancer, deep vein thrombosis, venous thromboembolism, D-dimer, ultrasonography

Introduction

There is a strong causal-consequential bond between carcinoma and venous thromboembolism (VTE). It was first pointed out by dr Armand Trousseau (in 1865) who announced a high incidence of deep vein thrombosis (TDV) within patients with gastric cancer. As a unique prediction, Trousseau later suffered from inexplicable TDV and died from gastric cancer. Nowadays, malignancy is widely accepted as a predisposed factor in TDV development.

Frequency of TDV within malignant patients is estimated to 15-25%, whereas a risk of lung embolism (LE) is increased twice to three times within these patients. TDV is found within almost 50% of patients who have pancreatic cancer and within

25% of patients who have lung cancer, where more than a half of cancers, comlicated by TDV episodes, belong to gastrointestal and urogenital systems.

Active cancer is responsible for almost 20% of new VTE cases, where the most frequent cancers within VTE patients are found in stomach, lung, breast, colon, rectum, and prostate gland. In addition to it, they represent the biggest problem for developed countries. However, some cancers are closely connected to a high risk of VTE appearance. This group, above all, includes malignant brain tumors and adenocarcinoma of ovaries, pancreas, colon, stomach, lungs, and kidneys. Intrahospital mortality is twice to three times higher when we compare patients with carcinoma who suffer from TDV and malignant patients who do not have this kind of complication. Presence of VTE makes the prognosis of patients with carcinoma a lot more serious, probably due to an increase of mortality and the fact that VTE appearance indicates more aggressive type of tumor.

In addition to prolonged immobilisation which is a well-known risk factor of thrombogenesis, malignant patients have a highlighted hipercoagulated status as a result of releasing tumor tissue factor and cancer procoagulant. It is proved that adenocarcinoma of colon, pancreas, stomach and others release tissue factor, that can activate coagulated cascade and predispose some malignant patients to TDV development. Due to a direct activation of X factor, cancer procoagulant is also considered to be a potential initiator of coagulated cascade within malignant patients and, therefore, creates conditions for forming a thromb.

The aim of the research has been a precise evaluation of frequently different clinical forms of TDV within patients with different types of digestive malignancy, as well as possibilities of early revelation of clinically serious and asymptomatic TDV forms with its aims to treat and prevent from its complications.

Methods

Using prospective clinical analysis all patients were included who were in the Clinic for gastroenterohepatology of the Clinical centre of Serbia, Belgrade, starting from 30th June 2009, to the end of December 2011, where different types of digestive malignancy were discovered. Each patient who fulfilled one or more exclusive criteria was excluded from the study: 1) previously objectively diagnosed TDV of ipsilateral leg without documented recanalisation; 2) suspected TDV, with clinically suspected concomitant of lung embolism; 3) treatment with anticoagulant therapy within month in duration over 48 hours; 4) unilateral amputation of lower limb; 5) obvious alternative symptomatic cause with clinical characteristics that do not respond to TDV; 6) disappearance of symptoms before more than 72 hours; 7) resistance and impossibility to allow a permission for analysing and following; 8) problematic follow-up of the patients due to geographic reasons, as well as 9) patients who had been operated before the follow-up period was finished.

The number of examinees has been reduced for those who suddenly died during the follow-up period and for patients who developed CVI, massive myocard heart attack or syndrom of multiorganic insufficiency. By a method of a random choice, all available patients have been classified into two groups (examined and control group).

All available patients have been exposed to a regular clinical level check (score) of TDV clinical probability, using contemporary clinical method by Wells. Patients who have had score < 2 are classified into a category of <<unlikely>> TDV, whereas those who had score >_ 2 are classified into <<likely>> TDV category. Firstly, D-dimer test has been done (using << D-dimer Hemosil IL>> reagent) to all of the patients of the examined group who had <<unlikely>> TDV category. Negative result of D-dimer test within these patients definitely excluded possibility of TDV. Ultrasonography of lower extremity magistral veins is done in cases of positive D-dimer test, where one can definitely find an answer to a question whether patients have TDV or not. Patients classified into category of <<likely>> TDV are firstly checked by ultrasonography, where positive analysis gave a definite confirmation of the illness. D-dimer testing is carried out in cases of negative ultrasound analysis. Negative result of the test excluded TDV within above mentioned patients, whereas the positive result indicated carrying out ultrasound within 2-3 days.

TDV diagnosis is only done by ultrasound within patients in control group. TDV has been

excluded by a negative ultrasound analysis within patients with <<unlikely>> TDV. In cases of negative ultrasound analysis within patients with <<likely>> TDV, a control ultrasonography has been done after 2-3 days, where an illness has been definitely confirmed or excluded.

Follow-up of examined patients has been maximally reduced to 10 days due to a permanent exposition of patients with digestive malignancy, a condition of uncontrolled hipercoagulability and possible initiation of new TDV, and necessary surgeon treatment or oncology therapy as well. After expiry date, all patients were examined by ultrasonography with its aim to evaluate a reliability of used diagnostic protocol.

Results

345 patients with digestive malignancy have been initially included in a study, where a distribution of patients according to a primary localisation of carcinoma and estimated clinical probability of TDV, has been shown in Table 1. The same chart shows the number and the structure of excluded patients.

Total of 49 patients with different localisations of digestive malignancy have been excluded from the study, where the reasons for their exclusion are shown in Table 2.

Out of 296 examined patients, 34 (22.97%) examinees from D-dimer group and 29 (19.59%) examinees from control group have been confirmed VTE with total prevalence of 21.28%. (Table 3).

Table 2. Reasons for exclusion of the patients from the study

Reasons for exclusion of the patients	Number
-Sudden death During the follow-up period	2
-CVI	1
-Massive myocard heart attack	2
-Shortly expected lifetime	9
-Rejected/not able to give approval**	6
-Geographical unavailability	4
-Over 72h since symptomatic resolutions	2
-Previous EP/TDV*	1
-Symptoms suspected to lung embolism	2
-Patients on anticoagulant therapy	2
-Allergy to contrast	1
-Incomplete follow-up	17
-Total	49

** Previously objectively diagnosed LE or TDV of ipsilateral leg without documented recanalisation*
***Refused or mentally unabled for valid approval*

Table 3. Distribution of VTE according to groups within examined patients with digestive malignancy

Study groups	Examined patients n/%	VTE*
		n (%; 95% CI)
D-dimer group	148/50	34* (22.97; 16.93-30.38)
Control group	148/50	29 (19.59; 14.00-26.72)
Total	296/100	63 (21.28; 17.00-26.30)

**11.48% regarding the total number of examined patients*

11 control group patients from the subgroup <<unlikely>> TDV have survived VTE (19.3%, 95%CI=11.13-31.34), where 6 have survived it during 10 days of the follow-up period, which is more

Table 1. Distribution of patients according to primary localisation of carcinoma and estimated clinical probability of TDV

Localisation of digestive malignancy	Starting number of patients	Number of excluded patients	Number of suitable patients	<<PTP score>>	
				< 2	≥ 2
Ca ventriculi	79	10	69	26	43
Ca pancreatic	73	12	61	21	40
Ca colonis	69	9	60	29	31
Ca recti	77	13	64	25	39
Ca hepatic	47	5	42	14	28
Total	345	49	296	115	181

«PTP score» (pre test probability score)

than a half (54.5%) of the total number of registered TDV in this group, i.e. 10.53% of examined patients of control group with <<unlikely>> TDV (95%CI=4.92-21.13). The above mentioned case of LE has been confirmed by perfused scintigraphy.

Out of 91 control group patient classified into subgroup of <<likely>> TDV, 18 patients (19.78%; 95%CI=12.89-29.11) have survived VTE, where 6 patients (33.3% of the total number of registered TDV in the group) have been discovered TDV on the day of the check, which represents 6.6% of examined patients with <<likely>> TDV (95%CI=3.05-13.64).

TDV has been discovered within 7 patients on the ultrasound check (repeated 3 days after the first check), whereas 5 patients have suffered VTE (4 TDV and 1 LE) during 10 days of the follow-up period. It represents 27.8% of the total number of registered TDV in the group, i.e. 5.5% (95%CI=2.37-12.22) of the total number of examined patients with <<likely>> TDV (Table 4).

During 10 days of the follow-up period, 11 new patients with VTE have been discovered in the control group (7.43% of the total number of examined patients; 95%CI=4.20-12.82), i.e. 8.46% (95%CI=4.79-14.52) out of 130 control group patients, where TDV has been initially excluded. Two cases of lung embolism (18.2%) out of 11 above mentioned patients with VTE have been registered and confirmed by perfused scintigraphy.

Considering a prevalence of TDV in the control group, differences between patients with highly probable and less probable thrombosis with 0.48% (95%CI=0.36-0.64) is not statistically significant (x2=0.01; p=0.927 n.s.) (Chart 4). Six patients from the control group with <<unlikely>> TDV have suffered VTE (one in form of lung embolism) during the follow-up period (54.5% all VTE in this group; 6/11), whereas other cases of TDV have been registered on the day of the check (45.5%). Five patients out of the total number of examined patients from the control group, have suffered VTE during the follow-up period (27.8%; 5/18), whereas 13 other patients have been detected TDV during the first check, i.e. on the US (72.2%). Difference in the structure of the registered thromboembolism regarding the diagnostic time is not statistically significant (Fisher's exact test=0.114; p> 0.05).

148 patients of D-dimer group who have finished follow-up have been classified into categories of <<unlikely>> (58, i.e. 39.2%) and <<likely>> TDV (90, i.e. 60.8%) (Chart 5.). TDV has been confirmed within 13 patients from the subgroup <<unlikely>> TDV (22.4% of the total number of examine patients of the subgroup; 95%CI=13.59-34.66), which was verified on the day of the check.

Table 4. Distribution of VTE in the control group in the aspect of the clinical probability of TDV

Control group	Examined patients n/%	VTE (n/% in the group according to risk)			
		On the day of the check	Control US	Follow-up	Total
«Unlikely» TDV	57/38.5	5/8.77	0	6/10.53	11/19.3#
«Likely» TDV	91/61.5	6/6.6	7/7.7*	5/5.5	18/19.78#
Total	148/100.0	11/7.43	7/4.7*	11/7.43**	29/19.6
X2=0.01; p=0.927 P>0.05#					

** 8.23% out of 85 control US in the subgroup*

Table 5. Distribution of VTE in D-dimer group, in aspect of clinical probability of TDV

D-dimer group	Examined patients n/%	VTE* (n/% in the group according to risk)			
		On the day of the check	Control US	Follow-up	Total
«unlikely»	TDV58/39.2	13/22.4	0	0	13/22.4#
«likely» TDV	90/60,8	9/10	11/12.2*	1/1.1	21/23.33#
Total	148/100,0	22/14.86	11/7.43*	1/0.67**	34/22.97
X^2 =0.02; p=0.896 n.s.#					

**23.4% out of 47 totally done US checks in the subgroup*

***0.87% (95%CI= 0.15-4.76) out of 115 initially excluded TDV of D-dimer group (follow-up)*

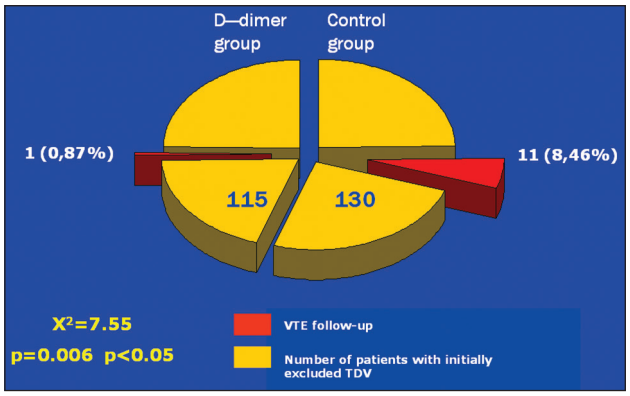
During the follow-up period there has not been registered any case of VTE in this subgroup (Table 5).

21 case of TDV (23.3% examined patients of <<likely>>TDV; 95%CI=15.80-33.05) has been registered within 90 patients of D-dimer group classified into <<likely>> category of TDV, where TDV has been verified during initial analysis within 9 patients (10% of the total number of examined patients of <<likely>>TDV, 95%CI=5.35-17.92). TDV has been registered on the control ultrasound within 11 patients (12.2% of the total number of examined patients, 95%CI=6.96-20.57), i.e. 23.4% of 47 done US checks in this subgroup).

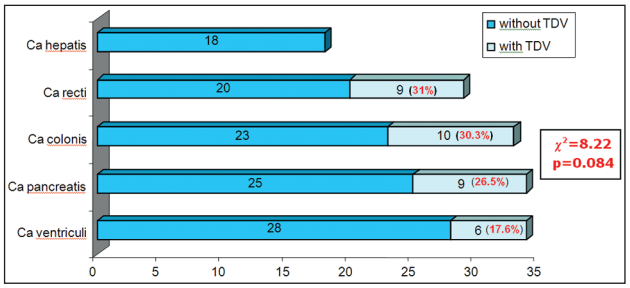
TDV has been registered within one patient from the subgroup during the follow-up period, which is 1.1% (95%CI=0.20-6.03) regarding total number (90) of patients from the subgroup, i.e. 0.67% (95%CI=0.12-3.73) regarding all the examined patients of D-dimer group, i.e. 0.87% (95%CI=0.15-4.76) regarding 115 patients from D-dimer group, where TDV has been initially excluded.

During 10 days of the follow-up period, 11 cases of VTE (8.46%; 95%CI=4.79-14.52) have been registered, where in 2 cases (18.2%; 95%CI=5.14-47.7) lung embolism has made some complications. Nevertheless, in D-dimer group, only 1 case of TDV has been registered (0.87%; 95%CI=0.15-4.76) out of 115 initially excluded TDV.

There is statistically significant difference between D-dimer and control group regarding the rate of different VTE manifestations during the follow-up period (0.87% (1/115) to 8.46% (11/130); c^2 test=7.55; p=0.006; p<0.05) (graph 1), where 95% of interval trust in difference to 7.59% amounts to 7.09-8.13.



Graph 1. Frequency of VTE in the follow-up period, regarding the number of patients with initially excluded TDV



Graph 2. Frequency of different localisations of digestive carcinoma in D-dimer group, comlicated by VTE development

Graph 2 shows frequencies of different localisations of digestive malignancies in D-dimer group, complicated by VTE development. The highest frequency of VTE is marked within patients with carcinoma of colon (30.3%) and carcinoma of rectum (31%). According to the frequency, patients with pancreatic carcinoma (26.5%) are on the third place. People suffering from gastric cancer are exposed to thrombotic complications in 17.6 % cases. Significant difference is not marked in VTE distribution within mentioned localisations of malignancy (c^2=8.22 p=0.084 n.s.). Absence of VTE within patients with liver carcinoma can be found in the same graph.

Discussion

Patients with malignant diseases are exposed to a high risk of VTE development responsible for heavy consequencies that, above all, indicate necessary long-term anticoagulant therapies, possibility of hemorrhage complications and constant risk of VTE recurrence as well.[20-22] It is confirmed that VTE which is present in the field of malignancy, significantly affects morbidity and mortality of these patients.[11-14-23]

The risk of recurrent VTE is twice to three times higher within patients with carcinoma than within patients who do not have any cancer.[23-24] Presence of TDV makes the prognosis of patients with cancer a lot more serious probably due to a higher rate of mortality and the fact that TDV indicates a more aggressive type of tumor.[25-29] Death within 9% of these patients can be directly assigned to thromboembolism, however, in most cases one cannot put a sign of equality between

thrombosis and death. In numerous studies VTE is shown as a sign of bad prognosis within these patients. Malignant patients who develop VTE are exposed to a high risk of earlier death, tumor progress and reduction of long-term survival.[30-31]

A study that has evaluated 4 458 patients from 115 centres of the USA, have shown that VTE is significantly independent sign of an early mortality and disease progress within malignant patients and that, therefore, it can be used in a short-term prognosis within malignant patients.[32]

It is assumed that a hipercoagulated condition of a surrogate aggressive cancer biology is responsible for an undesirable prognosis, even within patients in the earlier stadiums of disease.[33-34] Newer information indicates that activation of coagulation accelerates the growth of tumor and angiogenesis and, furthermore, confirms a connection between hipercoagulant condition and undesirable cancer prognosis.[35-36]

Diagnostic protocols used in the research have shown concerningly high incidence of VTE within patients with digestive malignancy in the control (19.59%) and in the examined group (22.97%) as well, with total prevalence of 21.28%. Such a high incidence of VTE that significantly exceeds information from earlier studies and confirms concerning evaluations of some authors,[3-9-31] is a result of highly sensitive diagnostic protocol which is used in the research.

The highest frequency of VTE is verified within patients with cancer of colon (30.3%) and cancer of rectum (31%). Smaller frequency of VTE is found within patients with pancreatic cancer (26.5%). Those who have suffered from gastric cancer have been exposed to thrombotic complications in 17.6% cases. Difference in the distribution of VTE within above mentioned localisations of carcinoma has not been statistically significant (c^2=8.22; p=0.084; n.s.).

Such a concerning VTE frequency whose appearance vitally deteriorates a course and prognosis of malignant disease, imperatively indicates a necessity of an early detection of phlebothrombosis, especially its asymptomatic and untypical varietes, and protective measures of primary thromboprofilaxy as well, in all cases of detected cancer. This research has verified VTE frequency of 8.46% within the control group during the follow-up period, which has been impermissibly high in comparison to the results of similar research carried out within populations who have not got any cancer.[1-19-37] Such a high percentage of VTE shows an unreliably used protocol of ultrasound diagnosis within patients with cancer.

In D-dimer group where D-dimer and ultrasound diagnosis has been combined according to strictly defined algorhythm, above mentioned frequency is significantly lower and amounts to only 0.87% regarding the total number of the patients from the group, where TDV has been initially excluded. During the follow-up period, confirmed difference of VTE frequency between D-dimer and control group is statistically significant (c^2 test=7.55; $p< 0.05$) and highly affirmative for the diagnostic protocol that is used in D-dimer group.

Conclusion

Concerning VTE frequency within patients with digestive malignancy requires imperative usage of D-dimer diagnostic protocol in its early detection and regular usage of thromboprofilaxy in all cases of detected colorectal, pancreatic and gastric cancer.

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

Key words: Camera ready paper, Journal, ksdh.

Introduction

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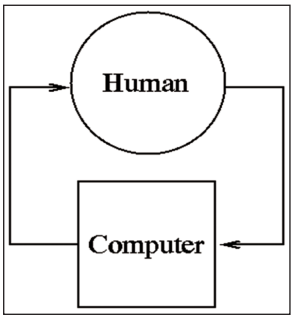

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

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