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Investigation of the wound healing effects of Chitosan on FGFR3 and VEGF immunlocalization in experimentally diabetic rats

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Abstract

Chitosan is a naturally occurring substance that stimulates correct deposition, assembly and orientation of collagen fibres in extracellular matrix components in wounds and promotes migration of inflammatory cells. Fibroblast growth factor (FGF) is one of the most important growth factors playing crucial roles in angiogenesis and wound healing. Biologically, it acts via binding to the cellular surface receptors. FGFR3 is one of the most important receptors. Therefore the aim of the present study was to investigate, histologically and histochemically, the effect of chitosan on wound healing in experimentally diabetic rats divided into four groups. When compared to the diabetic and the control groups, chitosan group had more inflammatory cells, endothelial cells, newly formed blood vessels and reticular – collagen fibres in the wound healing area from the third day of operation. Moreover, in Chitosan Group, stronger VEGF and FGFR3 immunolocalizations were evident and all steps of wound healing process were more regular. FGFR3 antibody used in this study had been tested only on diabetic wound healing. In conclusion, we have concluded that application of chitosan was essential to accelarate wound healing process in diabetic patients.

Key words: Diabetes, wound healing, chitosan, VEGF, FGFR3 .

Introduction

Diabetes mellitus is a chronic hyperglycaemic disorder (1). The prevalence of diabetes has increased tremendously and diabetic complications have become a serious health concern worldwide. Impaired-wound healing is one of these complications (2, 3, 4). Lack of cellular and molecular signals required for normal wound-repair process such as angiogenesis, granulation tissue formation, epithelization, and remodelling may be the major factors in poor wound healing in diabetes mellitus (3-7). Animal models are useful in studying early changes in diabetes. It has been observed that streptozotocin or alloxan might destroy β cells (8).

The healing of a wound requires a well-orchestrated integration of complex biological and molecular events of cell migration, proliferation, and extracellular matrix (ECM) deposition (9). Each phase is modulated by a vast array of cytokines and growth factors, which form an elaborate communication network co-ordinating the healing process. New understandings in the complexities of wound healing, and particularly the role of growth factors, are enabling clinicians to manage superficial wounds such as skin flaps and even the most difficult-to-heal wounds more effectively, but are deficient in diabetic wounds (3, 5-7). Multiple factors can lead to impaired wound healing in diabetic animals and patients. One of the important factors is that diabetic animals and patients do not produce enough growth factors (vascular endothelial growth factor; VEGF, fibroblast growth factor; FGF) and growth factor receptors (fibroblast growth factor receptor 3; FGFR3) (10-12).

Biomaterials are mostly polymers and are used in artificial organ production in contemporary medicine. The other areas for hydrogel usage are artificial tendon production, as bioadhesives in wound repair, as artificial kidney membrans, as artificial skin and biomaterial in plastic surgery. There are many monomers used in biomaterial production. In our previous studies, we investigated whether some monomers used in biomaterial production such as acrylamide, metacrylamide, N-isopropylacrylamide, acrylic acid, 2-hydroxyethyl methacrylate, 1-vinyl-2-pyrrolidone and ethylene glycol had cytotoxic effects and induced apoptosis or

not spinal cord. Immunolocalization of glial fibrillary acidic protein (GFAP) was olso determined, and it was evaluated by using semi-quantitative morphometrical techniques. The cytotoxicity of monomers on cultured fibroblastic cell lines was also examined *in vitro* (13-16). Chitosan, which is a poly D-glucosamine, is a deacetylated derivative of chitin (17). Chitosan and its oligomers are well known for their interesting biological properties which have led to various applications (17-20). Chitosan stimulate correct deposition, assembly and orientation of collagen fibers in extracellular matrix components in wounds. Moreover, histological findings indicate that chitosan membrane stimulates migration of inflammatory cells such as polymorphonuclear leucocytes, macrophages and fibroblasts. Thus it promotes granulation and cellular organization (17, 21-23).

Vascular endothelial growth factor (VEGF) is a multifunctional growth factor produced by endothelial cells, fibroblasts, smooth muscle cells, trombocytes, neutrophils and macrophages. Its function is to elicit proliferation, migration and differentiation of the said cells (24-26). Studies on wound healing process in experimentally diabetic animals have shown that several growth factors, including VEGF, are dramatically decreased (27-29). It has been suggested that administration of VEGF-A *via* protein or gene transfer methods increases granulation tissue formation, angiogenesis and matrix deposition in experimentally diabetic mice (27).

Fibroblast growth factor (FGF) demonstrates strong mitogenic properties in fibroblasts, osteoblasts, smooth muscle cells, endothelial cells, chondrocytes and melanocytes (27-30). It is one of the most important growth factors playing crucial roles in embryonic development, angiogenesis and wound healing. Biologically, it acts via binding to the cellular surface receptors belonging to the tyrosine kinase receptor family. Four of such surface receptors (FGFR 1-4) have been identified up until now (31-33). It has been clearly demonstrated in previous studies that FGFR3 is localized in the suprabasal region of epidermis, in the inner epidermal root sheat of hair follicles, in the smooth musle cells of blood vessels of the normal skin tissue whereas FGFR3 is immunolocalized in the suprabasal and basal layers of epidermis, around the blood vessels of the granulation tissue, in fibroblasts and inflammatory cells of the skin during wound healing process (33). However, the number of histopathological studies on VEGF immunolocalization is still limited while there exist no studies on FGFR-3 in wound healing process in diabetic subjects.

Therefore the aim of the present study was to evalute immunolocalizations of vascular endothelial growth factor (VEGF) and fibroblast growth factor receptor-3 (FGFR-3) in chitosan treated normal and experimentally diabetic rat skin during wound healing process and to study contribution of chitosan on wound healing process in diabetic conditions.

Materials and methods

Animals

In the present study, 51 Wistar albino rats reared in the Experimental Animal Laboratory, Cumhuriyet University, Sivas, Turkey and weighing 250-300 g were used. Animals were divided into four groups. While the control group had 6 animals, other groups had 15 animals all of which were fed with food and tap water *ad libitum*. All animals were treated under the guidance of Local Ethics Committee of Experimental Animals, Cumhuriyet University Sivas, Turkey. All the treatment procedures employed in the present study were approved by the same committee too.

Experimental Groups

An incision was made on the back of each animal in all groups.

Diabetic + Chitosan Group (DC) (n=15): Chitosan (Sigma, USA) was applied on the incision area every day.

Diabetic + Acetic Acid Group (DA) n=15): Only acetic acid was applied on the incision area every day.

Diabetic + Control Group (DO) (n=15): Citrate buffer was applied on the incision area every day.

Control Group (C) (n=6): Diabetes was not induced and betadin was applied on the incision area every day.

Experimental Diabetes Induction Procedure

Rats were not fed for overnight and and their blood glucose levels were measured in the

following morning (Lever Check TD-4222). Those having a blood glucose level between 80-100 mg/dl were regarded as non-diabetic. In order to induce diabetes mellitus, rats of either sex were given a single dose of 60 mg/kg streptozotocin (STZ) in 0.1 M citrate buffer, pH: 4.5, (Sigma Chemical Co., St Louis Missouri, USA) intraperitoneally (34, 35). Fourty eight hours after STZ injection, blood glucose levels were measured in samples obtained from the tail veins. Animals having a blood glucose levels above 250 mg/dl were regarded as diabetic. Rats had free access to food and water after STZ administration.

Preparation of Chitosan

In order to prepare 0.8 % Chitosan solution, 1 g Chitosan was dissolved in 100 cc 1% acetic acid and mixed for a few hours (36). The solution was calibrated to have a pH of 5.5. Solution was kept under UV light overnight for sterilization and to avoid bubbles. At the end of those processes, a sterile gel Chitosan was obtained to use in the experiments.

Surgical Procedures

All the animals received intramuscular injections of 90 mg/kg ketamine hydrochloride and 3 mg/kg xylazine hydrochloride into the left front leg muscles. The rats were anesthesized but allowed to breath spontaneously during the surgical procedure. In order to prevent postoperative pain, 4 mg/kg rimadyl was injected subcutaneously for 3 days. Using a surgical blade No. 10, two cm long full thickness incisions (37) were made at the back of rats. The wounds were not closed throughout the experiments. Chitosan, acetic acid and betadine were applied to the incision area every day and on the 3rd, 7th and 14th days after operation. 5 animals from each group were sacrificed by injecting a high-dose (200 mg/kg) sodium penta-barbital intraperitoneally. Tissue samples were obtained from the incision area to conduct light microscope and immunohistochemial investigations.

Light Microscopy

Skin samples obtained from the wound area were fixed in 10% buffered neutral formaline for 48 hours and blocked in paraffin after routine histological dehydration procedures. For immunohistochemical investigations, two - three μm thick tissue sections were taken by a Leica RM 2125 RT microtome. Sections were stained immunohistocemically for VEGF and FGFR3 and convenient fields of views were photographed using Olympus BX51 (Tokyo, Japan) photomicroscope.

Immunohistochemistry

For immunohistochemical staining, the deparaffinized and rehydrated tissue sections were inactivated using endogenous peroxidase by incubation in 3% H_2O_2 for 10 minutes. To recover antigen, these sections were put into EDTA solution (pH 8.5) and heated in microwave oven twice. Slides were then washed in PBS (pH7.2-7.6) twice. Non-specific binding sites were blocked in Ultra V Block (Lab vision, USA) solution for 20 minutes. After the redundant liquid was discarded, sections were incubated in primary antibodies (VEGF Ab-1 and FGFR3, Lab Vision USA) at room temperature for 1 hour and washed in PBS. Slides were then incubated in biotinylated secondary antibody (Lab Vision, USA) for 20 minutes and washed in PBS which was followed by incubation in streptavidin-HRP (Lab Vision, USA) for 20 minutes and by washing in PBS. Antibody binding sites were visualized by incubation with an AEC chromogen (Lab Vision, USA) solution. Slides were counterstained for 1 minute in hematoxylin and then dehydrated in sequential ethanol series for sealing and microscopic observations.

Results

Histological features stained immunohistochemically, were evaluated semi-quantitatively and the results are shown in Tables 1 and 2. In Chitosan Group, immunolocalization of vascular endothelial growth factor (VEGF) (Table 1, Figures 2, 4, 6) revealed a strong expression in the epithelium close to the wound region, in the healing area, in the sebaceous glands and around the blood vessels on the 3rd day; however, its immunolocalization decreased gradually on the 7th day and it was rather weak on the 14th day when compared to the other groups. Immunohistochemical staining by FGFR3 (Table 2, Figures 8, 10, 12) in Chistosan Group revealed a very strong expression on the 3rd day, a rather weak expression on the 7th day and a strong expression on the 14th day in the epi-

thelium close to the wound region and around the hair follicles, sebaceous glands and blood vessels when compared to the other groups.

Discussion

Diabetes is a chronic metabolic disease affecting the majority of the world population. While the number of people having diabetes for several reasons has been increasing, complications observed in diabetes have been increasing too (1-4). One of the most important complications seen in diabetic patients is the impaired wound healing (38, 39).

At the present, several subsidiary biomaterials have been used for a better wound healing therapy and chitosan is one of them. Chitosan is a natural polymer made by chitin. Chitin is the most abundant polymer after cellulose and it is present in the cell wall of sea shells and mushrooms (17,18, 40). Chitosan, a biodegradable and biocompatible polymer, is an important and indispensable biomaterial in pharmacology and in medicine since it is nontoxic and causes no allergy or irritation. At the same time, through accelarating the wound and bone healing, Chitosan is a haemostatic, antibacterial and antifungal immune system stimulant (19-21, 41).

Wound healing process occurs around three main events; haemostasis and inflammation, new tissue formation and remodelling (30, 42, 43). These events occur not in a particular order but in a complicated manner (30, 42-44). During wound healing process, angiogenic growth factors such as vascular endothelial growth factor (VEGF), placental growth factor (PGF), acidic and basic fibroblast growth factor 1 and 2 (FGF1 and 2), fibroblast growth factor 3 and 4 (FGF3 and 4), FGF receptors, transforming growth factor α-β (TGFα-β), epidermal growth factor (EGF), hepatocyte growth factor (HGF), angiogenin, platelet derived growth factor (PDGF, granulocyte colony stimulating factor (GCSF), interleukin 8 (IL 8), tumour necrosis factor-α (TNF-α) and proliferin are important (45).

Vascular endothelial growth factor (VEGF) is a member of multifunctional growth factor family and has specific effects especially on endothelial cells. It binds to its receptors with a complex system thus regulates blood vessel formation (24-26, 45). During wound healing process, vascular endothelial growth factor (VEGF) is produced by several cells such as endothelial cells, fibroblasts, smooth muscle cells, trombocytes, neutrophils and macrophages. Its functions are proliferation, migration and differentiation of endothelial cells (23-25). In a previous study, VEGF immunoreactivity and its relationship with angiogenesis has been described in glioma cells (45-47). Recent studies have shown that VEGF may have effects on collagen deposition and epithelial formation (48). In the first phase of wound healing, VEGF stimulates the coagulant factors in the endothelial cells. Therefore, the trombocyte accumulation and adhesion occur (46-48). For blood vessel formation, immunolocalization of vascular endothelial growth factor is increased in inflammatory cells during inflammation period of the wound healing while it is also observed in fibroblasts, endothelial cells and macrophages during the following phases of the wound healing process (46-48). Blood vessel formation (angiogenesis) occurs on the 3rd day of the wound healing process. In order to attract metabolites and oxygen to the healing region, the number of capillaries increases in the granulation tissue (48, 49). Previous studies have clearly shown that keratinocytes around the wound, fibroblasts and macrophages in the wound region, macrophages in the wound region and growing blood vessels start to produce VEGF whose secretion reaches its maximum level on the third and the seventh days (49, 50). In the present study, VEGF immunolocalization was strong on the 3rd day while it gradually decreased on the 7th and 14th days in the control group. Previous studies have demonstrated that VEGF production is rather high between the 3rd and 7th days in wound healing area (48-50). Formation of granulation tissue, which contains fibroblasts, macrophages and endothelial cells all of which are essential for VEGF secretion, occurs on the third and seventh days of the wound healing process (50).Findings of the present study revealed that VEGF expression was low on the 3rd day, moderate on the 7th day and strong on the 13th day in the diabetes+control and in the diabetes+acetic acid groups. Like all other growth factors, VEGF production is suppressed in diabetic conditions (27, 28). VEGF secretion form keratinocytes and fibroblasts has been shown to be decreased in in diabetics (27). Altavilla et al. have suggested that oxydative stress causes impairments in VEGF secretion and regulation in diabetic pa-

tients (50). In a histopathological study on diabetic mice, Komesu et al. showed that the onset of inflammation phase was delayed in diabetic animals and chronic inflammation findings lasted longer in samples collected on the 1st, 3rd and 7th days (1). In our study, VEGF immunolocalization was low in the diabetes+control and in diabetic+acetic acid groups on the 3rd and the 7th days. However, VEGF immunolocalization was very strong on the 3rd day, strong on the 7th day and moderate on the 14th day in diabetic+chitosan group. Histopathological findings of previous studies have revealed that leukocytes and macrophages reach wound area faster in chitosan treated animals when compared to the controls (21-23, 51). Therefore, it can be suggested that chitosan might stimulate inflammatory cells and their growth factors in the wound area during the early phases of the wound healing process. In fact, chitosan attracts inflammatory cells and VEGF to the wound healing area during the early stages of the wound healing process (21, 51). Ueno et al. have suggested that chitosan accelarates granulation tissue formation (21). At the same time, chitosan activates fibroblasts in the granulation tissue, caused them to proliferate and accelerates extracellular matrix production (23). Therefore, the present study suggests that chitosan might accelerate the wound healing by increasing VEGF secretion despite the negative effects of diabetes.

It has been suggested that acidic fibroblast growth factor (aFGF or FGF1) and basic fibroblast growth factor (bFGF or FGF2) are potential angiogenic factors in diabetics just like VEGF (22-24). Fibroblast growth factor receptors also play key roles in the wound healing process. Four types of FGF receptors have been found in epidermal layers, muscles, blood vessels, fibroblasts, hair follicles, granulation and inflammation tissues of normal and wounded tissues during the wound healing process (31-33, 52). Takenaka et al. have evaluated normal and burned human tissue samples and suggested that FGFR1 and FGFR3 have strong expressions in the first stage of the healing process while these receptors has weaker expressions following the granulation tissue formation (33). On the other hand, they have also suggested that when FGFR1 and FGFR3 have weak immunolocalizations, FGFR2 and FGFR4 have strong expressions along with moderate FGFR1 and FGFR3 expressions towards the final stage of the healing process (33). However, there are no data on the FGFR3 expression in diabetic wound healing process. In the present study, the FGFR3 immunolocalization in the basal layer of epidermis, in the epidermal root sheath of hair follicles, around the blood vessels, in sebaceous glands and in the healing region were very strong on the 3rd day, moderate on the 7th day and strong on the 14th day in Chitosan Group when compared to the control and the other groups. Chitosan is very affective on the secretion of growth factors from the cells in all stages of wound healing process (17-23, 51). A similar finding, namely strong expression of FGFR3 in the wound healing area, was found in our study stoo. The present study showed that the healing process of full thickness incisions made in diabetic rats was affected positively when chitosan was applied. Besides having antibacterial and antifungal properties, Chitosan is a cheap and easily obtained natural polymer. Wound healing is a complex process including number of cells and growth factors. Due to being very a expensive and time consuming process, treatment of wounds in diabetic patients is very difficult. By demonstrating VEGF and FGFR3 expressions in the wound healing regions, the present study has clearly shown that application of chitosan, with its positive effects on wound healing process, helps to overcome the above mentioned difficulties.

Table 1. Semi-quantitative comparison of VEGF expressions on the 3rd, 7th and 14 day in the Control (C), Diabetic Acetic Acid+Diabetic Control (DA+DO) and Chitosa Groups (DC)

VEGF	C	DA+DO	DC
3rd Day	++++	++	+++++
5th Day	+++	+++	++++
14th Day	++	++++	++

+++++ very strong, ++++ strong, +++ moderate, ++ low, + very low

Table 2. Semi-quantitative comparison of FGFR3 expressions on the 3rd, 7th and 14th days in the Control (C), Diabetic Acetic Acid+Diabetic Control (DA+DO) and Chitosan Groups (DC)

FGFR3	C	DA+DO	DC
3rd Day	++++	+	+++++
5th Day	+++	+++	++
14th Day	++++	+++	++++

+++++ very strong, ++++ strong, +++ moderate, ++ low, + very low

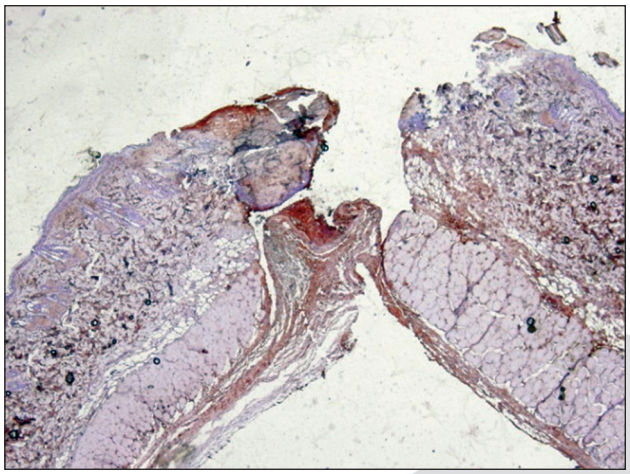

Figure 1. Control Group, on days 3 (X4), VEGF immunolocalization in the wound healing region

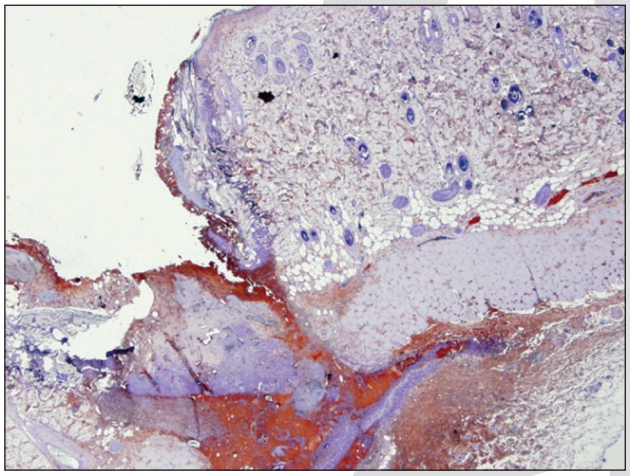

Figure 2. The VEGF immunolocalization on days 3 (X4), in the incision made and chitosan application group (DC)

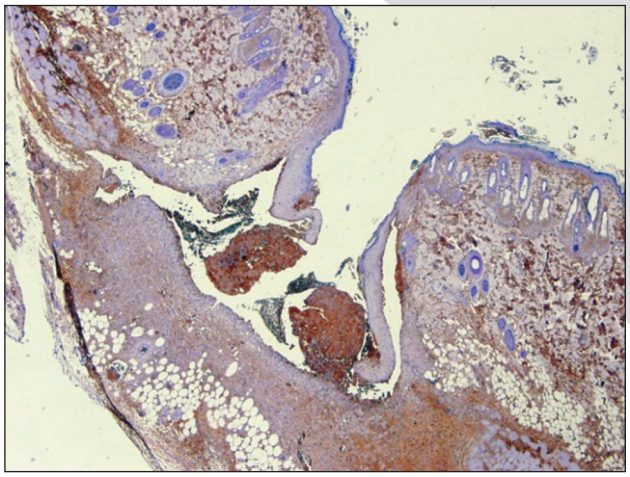

Figure 3. Control Group, on days 7 (X4), VEGF immunolocalization in the wound healing region

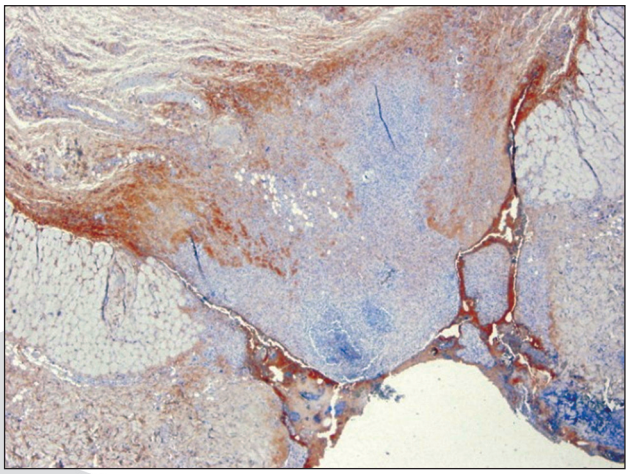

Figure 4. The VEGF immunolocalization on days 7 (X4), in the incision made and chitosan application group (DC)

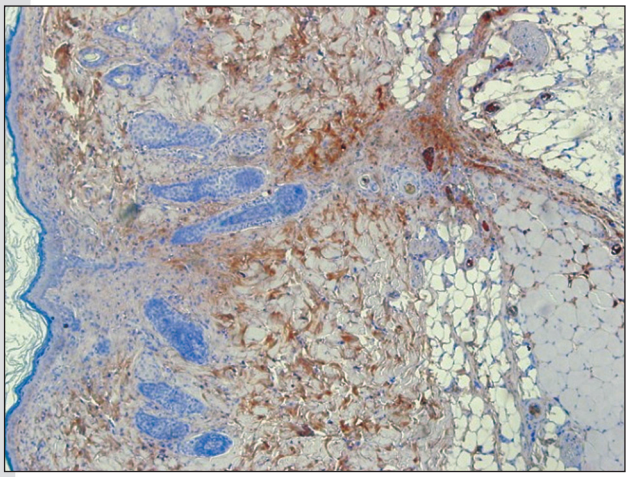

Figure 5. Control Group, on days 14 (X4), VEGF immunolocalization in the wound healing region

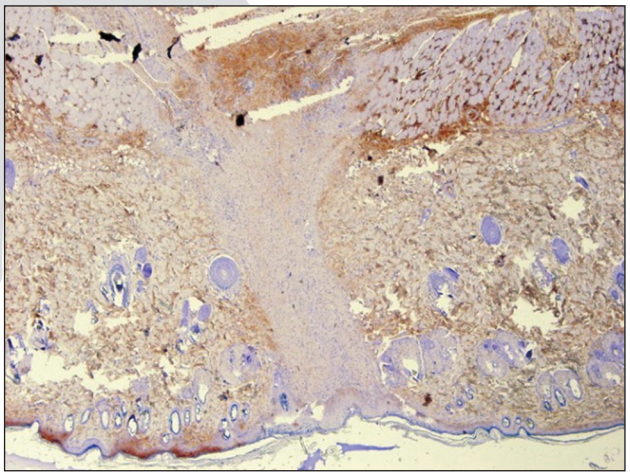

Figure 6. The VEGF immunolocalization on days 14 (X4), in the incision made and chitosan application group (DC)

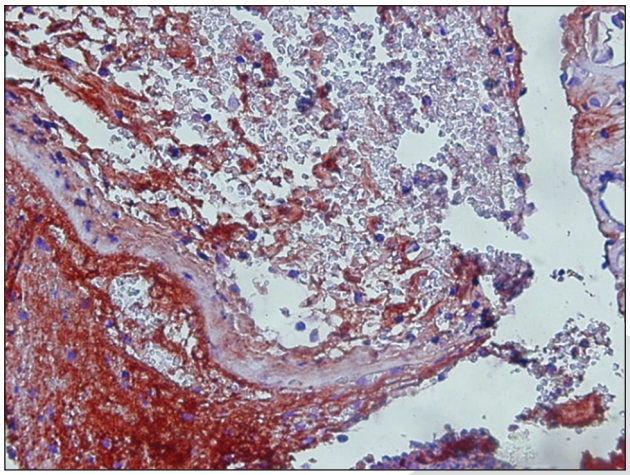

Figure 7. Control Group, on days 3 (X40), FGFR3 immunolocalization in the wound healing region

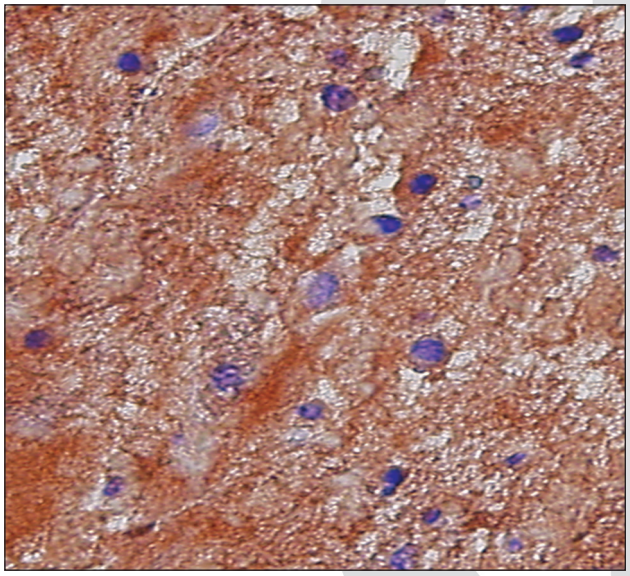

Figure 8. The FGFR3 immunolocalization on days 3 (X40), in the incision made and chitosan application group (DC)

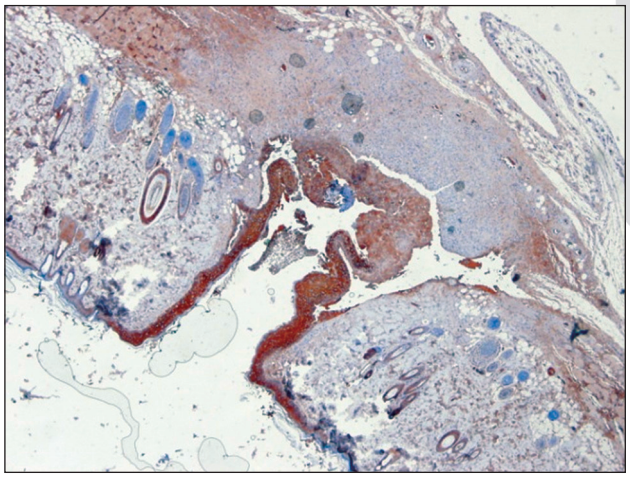

Figure 9. Control Group, on days 7 (X4), FGFR3 immunolocalization in the wound healing region

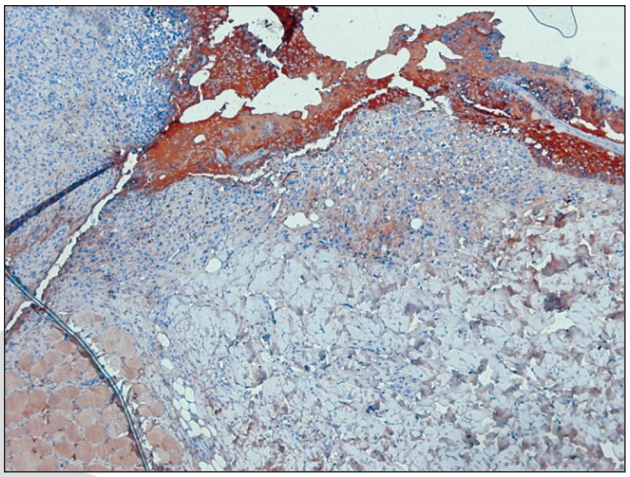

Figure 10. The FGFR3 immunolocalization on days 7 (X10), in the incision made and chitosan application group (DC)

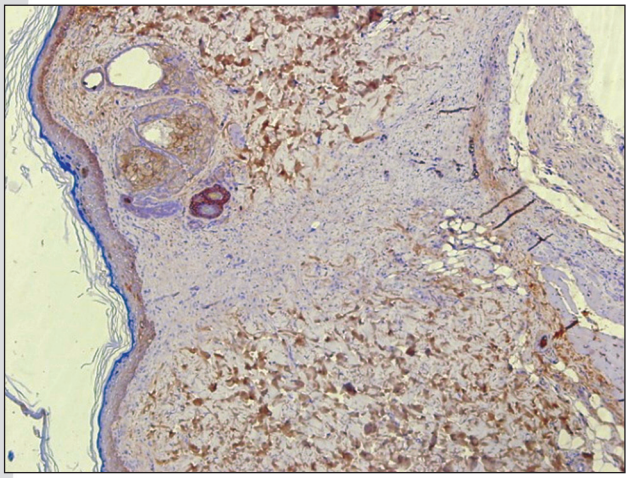

Figure 11. Control Group, on days 14 (X10),FGFR3 immunolocalization in the wound healing region

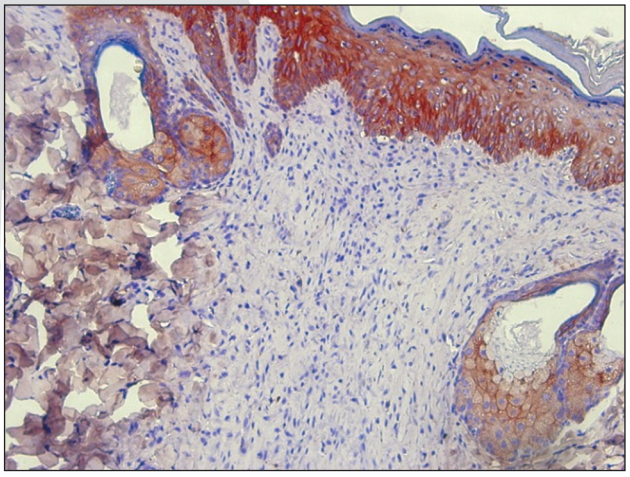

Figure 12. The FGFR3 immunolocalization on days 14 (X20), in the incision made and chitosan application group (DC)

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Prevalence and implications of Coeliac Disease in undetected adults compared to those diagnosed with irritable bowel syndrome (IBS) in Riyadh, Saudi Arabia

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Abstract

We conducted a study of 720 adults, half of them randomly selected as a control, for undetected coeliac disease of males and females, aged between 20 and 60 from students and employees in two Riyadh Colleges of Health Sciences.

We also compared the same number of 360 cases with a randomly selected group diagnosed with irritable bowel syndrome from Al-Iman Hospital at Riyadh, Saudi Arabia, within the same age range and similar mixed gender distribution.

The control consisting of undetected coeliac disease and the cases with Irritable Bowel Syndrome (IBS), both randomly selected, were tested for autoimmune antibodies to tissues transglutaminase(tTGAs) and endomysial autoantibody (EMAs) as serological predictors of coeliac disease and positive patients of coeliac disease were confirmed by histopathology test. The confirmed cases of coeliac disease were tested for iron deficiency anaemia, Vitamin D deficiency, osteoporosis and weight assessment.

In the undetected control the percentage of coeliac disease was found to be in the of 1.9%, slightly higher than the percentage world wide range in adults with undetected coeliac disease. However, in patients diagnosed as irritable bowel syndrome (IBS) the percentage was found to be almost five times to that of the control at 9.5%.

These findings might explain that given a considerable similarity of symptoms of coeliac disease and irritable bowel syndrome, can lead to the over-diagnosis of irritable bowel syndrome, or on the other hand misdiagnosis of coeliac disease.

Key words: Coeliac Disease (CD), Irritable Bowel Syndrome (IBS).

Introduction

Coeliac disease is an autoimmune multisystem disorder that occurs in genetically susceptible individuals in response to dietary gluten in wheat, rye and barley. Ingestion of gluten by susceptible individuals causes morphologic changes in small intestinal mucosa, leading to villous atrophy and ultimately to malabsorption. It has been considered an uncommon gastrointestinal disorder affecting mainly children, but according to recent worldwide records the disease has become a common systemic condition affecting individuals of all age groups (1).

In many Western countries, the disease affects approximately 1% of the population, but it has recently been shown that the true prevalence of coeliac disease is increasing over time.[1] The prevalence also increases by age within a country; for example, in Finland, the prevalence is 1.5% in children (2), 2% in adults (1) and 2.7% in the elderly (3). The prevalence in the general population of the United States is reported to be almost 1% (4). Several serological screening studies from Europe, South America, Australasia, and the USA have shown that approximately 0.5–1% of their populations may have undetected coeliac disease. The most consistent estimate reported from the largest population based studies shows the prevalence of undetected coeliac disease at approximately 1%. The prevalence is even higher among first and second degree relatives of individuals with coeliac disease (4). The symptoms and signs of coeliac disease vary from mild to severe, and some with coeliac disease can be asymptomatic for years or decades. The classical symptoms include the following: malabsorption; chronic diarrhoea; iron deficiency anaemia and weight loss. In children, short stature is a prominent symptom. In addition to gastrointestinal symptoms,

the disease has extra-intestinal manifestations, such as osteoporosis, dermatitis herpetiformis, neurological disorders, liver disorders, arthritis and obstetric problems (5). Coeliac disease is also associated with other autoimmune disorders, such as type one diabetes mellitus and autoimmune thyroid diseases (6).

Irritable bowel syndrome (IBS) is a chronic functional disorder of the gastrointestinal tract of unknown origin. The population prevalence in community surveys varies between 5% and 20% depending on the criteria used to define its presence (7), and the condition is more common in female and younger individuals (8). The natural history often follows a chronic relapsing-remitting course (9). Affected individuals report symptoms such as lower abdominal pain, diarrhoea, and abdominal bloating or distension (10). Symptoms reported by patients with coeliac disease also include bloating, abdominal pain, and chronic diarrhoea. In contrast to IBS, symptoms of coeliac disease may resolve if it is correctly diagnosed and gluten is excluded entirely from the diet (11).

Both irritable bowel syndrome and coeliac disease are conditions that share a common set of symptoms. The average time between seeing a physician because of symptoms and the ultimate diagnosis of coeliac disease is 12 months. Previous studies indicate that individuals meeting diagnostic criteria for irritable bowel syndrome might be at higher risk of having also coeliac disease compared to controls without irritable bowel disease (11).

One explanation for any changes in presentation could be that the natural history of the disease is changing, perhaps in response to changing environmental stimuli such as infant feeding practices in children or cigarette smoking in adults. A more likely explanation is that the ability to make the diagnosis has improved (both better tests and greater test accessibility) throughout the last 20 years with the development of accurate serological markers of the coeliac disease and increasing use of endoscopic biopsy techniques. Therefore, a much broader spectrum of individuals are being investigated for coeliac disease and consequently being diagnosed.

Material and Methods

Subjects and criteria

This prospective study was designed to screen 720 of case series and case-control studies in which randomly selected adults meeting diagnostic criteria for irritable bowel syndrome were chosen and serologic tests for coeliac disease were performed in all involved individuals accepted for the study. This study was performed over a one year period between September 2010 and September 2011,

An ELISA method based on the levels of tissue transglutaminase antibodies (tTGAs) allows diagnosis of coeliac disease with a high sensitivity and specificity. IgA anti-tTGA and Immunoglobulin A Endomysial antibodies (IgA EMA) show an excellent correlation confirming the enzyme as the coeliac disease autoantigen. This study was approved by the local Research Ethics at hospital.

Diagnostic criteria for irritable bowel syndrome included a physician decision, questionnaire data, or specific symptom-based criteria including those of Manning et al (10) ,the Rome criteria (12) or the scoring system of Kruis et al.(13) These could be supplemented by results of gastrointestinal tract examinations if these were performed in the individual studies. We considered EMAs, and tTGAs as valid serologic markers of possible coeliac disease. It was necessary to include distal duodenal biopsy to confirm coeliac disease in individuals with positive results of serologic tests to be acceptable for study. The eligibility criteria for study inclusion were as follows: (1) study participants were adults with a presumed diagnosis of irritable bowel syndrome according to physician decision , questionnaire findings, or normal al (14), Rome I, II, or III criteria, or the scoring system of Kruis et al (13); (2) the design was a case series or case-control study; (3) participants were randomly selected; and (4) all participants underwent serologic testing for coeliac disease and the results were recorded (EMAs, and tTGAs,) .

ELISA tests

Serum samples were centrifuged and stored at -30°C until analysis. They were first detected by visual CD-LIA for IgA antibodies to human tTG and gliadin. Then, positive samples were tested again for EMA known as anti-tTG antibody against transglutaminase and antigliadin antibodies (AGA) by commercially available enzyme-linked immunosorbent assay (ELISA; Euroimmune, Germany) as a second step. Thus, confirmation of all positive anti-tTG and anti- gliadin

tests by conventional ELISA tests was made before recommending a biopsy was done.

Small Bowel Biopsy and Histopathologic Evaluation

Small bowel biopsy (SBB) was recommended to all adult cases with positive anti-tTG and EMA/AGA results. SBB was performed with endoscope (Olympus GIF type P30®, Japan). Intestinal biopsy specimens were obtained from the second part of the duodenum. Biopsy specimens were fixed in formalin, embedded in paraffin, sectioned then treated with hematoxylin-eosin stain, and evaluated according to the Marsh criteria by a single expert pathologist who was blinded to the serology results and clinical information.

Other parameters

Iron deficiency: when serum ferritin levels of less than 18 ng/mL were measured using Axsym, Abbott (IL, USA).

Serum 25-hydroxyvitamin D [25(OH)D] concentrations were determined using the DiaSorin "25-OH Vitamin D TOTAL" competitive chemiluminescence immunoassay on the automated LIAISON® analyzer (MN, USA).

Assessment of Bone Density Using Dual Radiograph Absorptiometry (DXA*)*

Statistical Analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS v. 11.5) for Windows (SPSS Inc., Chicago, IL) software computer program. Chi-square and Student's t tests were used to compare results. A *p* value less than 0.05 was considered significant.

Results

Analysis of results

A total of 42 patients with coeliac disease were identified among all of the 720 candidates involved in this study, with different age group and gender. Seven individuals with coeliac disease were detected among the control of 360 (1.9%) compared to the 35 coeliac patients detected in 360 cases (9.5%) diagnosed with irritable bowel syndrome giving a 5 fold higher rate over the control.

A higher prevalence, about 40% of all coeliac disease patients, was noted among females in the middle age group of 20-39 years, , compared to 16 % of the males on the same age range (Table1).

The prevalence of coeliac disease in male subjects age group of 40-59 was 23% of the total coeliac disease patients compared to 19% of female on the same age range (Table1).

All of the 42 cases and controls were tested for serological markers of coeliac disease (tTGAs and EMA) at King Fahd Medical City Laboratory and were confirmed by histopathology diagnosis afterwards.

39 of the total 42 of cases and controls were found to be positive for both tTGAs and EMAs, two cases were found to be positive for tTGAs and negative for EMAs and one case found to be positive for EMAs and negative for tTGAs. However all of the 42 cases were tested by histological diagnosis, which confirmed the presence of coeliac disease in 42 patients.

Histopathology description of coeliac disease is still considered the gold standard and clear diagnosis of coeliac disease even, with negative serological antibodies of coeliac disease, with the conclusion that villous atrophy and crypt hyperplasia with cells infiltration in small bowel are the main pathology of coeliac disease.

After coeliac disease has been confirmed, the 42 patients were tested for relevant diseases associated with coeliac disease and the results were found as follows:

In the 42 cases diagnosed with coeliac disease, the implications of undetected coeliac disease were calculated as follow: there were underweight

Table 1. Distribution of age and sex ranges in the 42 patients found to be positive for coeliac disease among 720 individuals

No. Of Male Patients with coeliac disease	No. Of Female Patients with coeliac disease	Age Range
4	9	20-29
3	8	30-39
5	5	40-49
5	3	50-59

Table 2. Diseases Detected in 42 Patients with coeliac disease

No. of Patients	Underweight	Iron Deficiency Anaemia	Vit. D Deficiency	Osteoporosis	Dermatitis
18	Positive	Negative	Positive	Positive	Negative
12	Negative	Positive	Positive	Negative	Negative
7	Positive	Negative	Negative	Positive	Negative
3	Positive	Positive	Negative	Positive	Positive
5	Positive	Negative	Negative	Negative	Negative

patients (78.6%); iron deficiency anaemia was detected in 35.7%; Vitamin D deficiency in 71.4%; osteoporosis in 66.6% and dermatitis in 7%. (As shown in Table 2 and Figure 1)

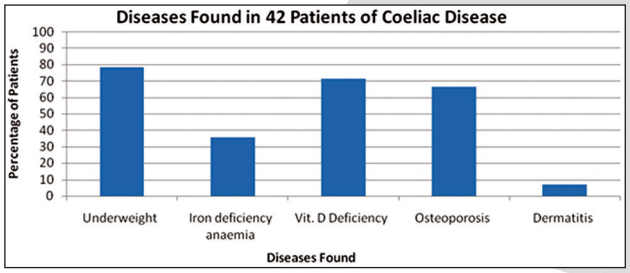

Figure 1. Diseases Found in 42 Patients of Coeliac Disease

Discussion

This study provides an estimate of the prevalence of serological markers and biopsy-validated coeliac disease in individuals diagnosed with irritable bowel syndrome and also the prevalence of undetected coeliac disease in adults.

The prevalence of positive serological findings with biopsy- diagnosed coeliac disease was found to be 1.9% in control undetected adults, while the prevalence of positive serological findings with biopsy-proven coeliac disease was 9.5% on the group of cases of irritable bowel syndrome patients.

The implications of recognising undetected coeliac disease in a general population are unclear because there are only limited reported data, from small selected case studies, on the morbidity and physiological characteristics associated with previously undetected disease. So far, few studies have been able to analyse the wide variety of socio-demographic and physiological factors with respect to undiagnosed coeliac disease, primarily due to low sample size. Most adult screening studies in the general population have identified only a small number of previously undiagnosed cases and have therefore been unable to examine any associations in comparison with the general population (15). Of the studies that have looked at the differences between clinically diagnosed disease and "screening" or previously undetected disease, most have focused on bone mineral density and anthropometric measurements. These findings suggest that people with undetected coeliac disease have a slight tendency towards low bone density and measurements in keeping with mildly subnormal nutritional status (16).

Analysis of a population based cohort from Cambridge, UK, showed that there were some negative health effects of undetected coeliac disease, for example mild anaemia and osteoporosis (17). However, the study findings implied a reduced risk of cardiovascular disease from the observations of lower body mass index, lower blood pressure, and lower serum cholesterol in individuals with undetected coeliac disease. This effect, of potentially large impact, requires confirmation and further epidemiological assessment.

The present study aimed to investigate the prevalence of coeliac disease among patients that fulfil the Rome III criteria for irritable bowel syndrome, referred to the gastroenterology section of Al-Iman hospital in Riyadh over the past three years.

Irritable bowel syndrome is prevalent in 15-25% of the world population, while the prevalence of coeliac disease is only 0.5-1% (18). There is, however, an overlap in the symptoms between irritable bowel syndrome and coeliac disease. Since the diagnosis of irritable bowel syndrome is based mainly on symptom assessment, there is a risk of coeliac disease patients being wrongly diagnosed as having irritable bowel syndrome. This risk is even higher in the two irritable bowel syndrome subtypes: irritable bowel syndrome-diarrhoea and irritable bowel syndrome-mixed. The situation is further complicated by the fact that the

abdominal symptoms of both coeliac disease and irritable bowel syndrome patients are triggered by the ingestion of wheat products. In coeliac disease patients, this is due to gluten allergy, while in irritable bowel syndrome; the effect is attributed to the long sugar polymer fructan in the wheat (18).

This study has demonstrated that there are considerable implications of undiagnosed coeliac disease including underweight, osteoporosis and nutritional deficiencies such as Vitamin D and iron deficiency.

The strong points supporting the conclusions of this study are:

- All the candidates were randomly selected including the control and the cases of irritable bowel syndrome.
- The control and cases of irritable bowel syndrome were shown to be positive for coeliac disease by testing for both EMAs and tTGAs antibodies and confirmed by histological diagnosis.
- The Laboratory used for the serological antibodies of coeliac disease has been accredited by the Canadian Association of Laboratory Medicine.
- Finally, the study used subjects from a wide range of age both genders.

The quality of this study had some limitations, which are:

- The number of controls and cases were relatively small in size.
- There were not enough available tests and detailed documentation of family, allergic and food history in the patients' notes.

Recommendations:

- All the patients admitted to the hospital with symptoms suggestive of irritable bowel syndrome should be seen at a gastroenterology consultant level with considerable work experience for a professional diagnosis of irritable bowel syndrome according to the worldwide criteria for example Rom III classification, to avoid over diagnosis of irritable bowel syndrome or misdiagnosis of coeliac disease.
- Due to the overlap of symptoms of irritable bowel syndrome and coeliac disease, it is beneficial to perform a serological test for coeliac disease, for any patient complaining of chronic lower abdominal pain and diarrhoea. This test is available, it is relatively inexpensive, and has high sensitivity and specificity for diagnosis of celiac disease.
- Any patients who have professional diagnosis of irritable bowel syndrome should perform immediate blood test of full blood count and erythrocyte sedimentation rate, and thyroid function as recommended by British Society of Gastroenterology.
- For confirmation of the above results and further implications of coeliac disease, much more research and epidemiological studies are needed with a sufficiently large number of cases, especially since there have been a very minimal amount of research in the fields of coeliac disease and irritable bowel syndrome in the Kingdom of Saudi Arabia.

Conclusion

The prevalence of coeliac disease among undetected adults was 1.9%, higher than the reported value worldwide, but lower than that reported in adults with coeliac disease in Finland. The high prevalence of coeliac disease found in this study of 9.5% among the cases diagnosed with irritable bowel syndrome strongly suggests that the similarity and overlap of symptoms of the two conditions very likley leads to the misdiagnosis of coeliac disease. Subsequently, the implications of this relatively common gastroenterology disease, according to our study and many recent studies, support the theory that coeliac disease is a disease of all age groups. In some countries, however, coeliac disease was found to be more common in adults and elders rather than children. We also discovered that coeliac disease is very common in middle-aged females.

Implications of coeliac disease, especially nutritional ones such as iron deficiency anaemia and Vitamin D deficiency caused by malabsorption, are alarming symptoms thus making the diagnosis of irritable bowel syndrome very unlikely in patients with chronic abdominal pain and diarrhoea. Serological testing for antibody markers of coeliac disease should be performed in any patient with severe chronic abdominal pain and diarrhoea to either confirm or exclude the diagnosis of coeliac disease.

On the other hand, histological investigations, should be carried out when patients are negative using serology tests. If the diagnosis of coeliac disease is confirmed at an early stage by serological tests, especially since nowadays these are readily available, they are inexpensive and have high sensitivity and specificity. If gluten-free diet is introduced immediately after the diagnosis of coeliac disease, the symptoms of the disease will be reduced considerably or entirely disappear, subsequently saving a lot of time and expenses for patients and health resource. Morover, early daignosis will also prevent any additional dangerous complications that could happen as a result of the coeliac disease.

The professional diagnosis of irritable bowel syndrome should only be done by expert consultant gastroenterologists with considerable experience, according to the worldwide criteria for the diagnosis irritable bowel syndrome and should included full blood counts, erythrocyte sedimentation rate determination and assessment of thyroid functions as recommended by the British Society of Gastroenterologist, in order to avoid overdiagnosing this common disease.

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Comparing the results of cut-off method, anti-mode method and mirror image method in estimating the prevalence of tuberculosis infection

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Abstract

Background and objectives: To estimate the prevalence of tuberculosis infection, PPD test is used commonly. However, there are different methods to interpret its results. We estimated the prevalence of infection using three different techniques in analysis the results of PPD test.

Methods: A tuberculin skin test survey was carried out in southeast of Iran among school children aged 6-9 years. Prevalence of TB infection was estimated from the reaction sizes of the tests using cut-off point, anti-mode and mirror image methods. Results were compared in all subjects, and in subgroups according to age, sex and BCG scar status.

Results: A total of 1708 students (85.5% participation rate) were tested and read with the mean (SD) age of 7.1(0.8) years. The estimated prevalence by cut-off point 10 mm, anti-mode and mirror methods among all children was 3.8% (2.9-4.8), 3.2%(2.4-4.1) and 5.1%(3.7-6.2) respectively. The most agreement was observed between the results of anti-mode and cut point methods (kappa=0.91, p<0.0001). The maximum agreement between the mirror method and cut point of 8 mm was observed.

Conclusion: The prevalence of tuberculosis infection using the traditional cut-off 10mm showed a lower estimation comparing to the results of the other methods. Considering the increasing challenges about the accuracy of the cut-point method, it is better to estimate the prevalence of TB infection by most sophisticated methodologies.

Key words: Tuberculosis, infection, prevalence, tuberculin survey**.**

Introduction

Nowadays, despite the implementation of effective tuberculosis control programs, tuberculosis(TB) continues to be a major health problem in the world [1] and is a leading public health concern in Iran as well.

A main target within 8th goal of the MDG (Millennium Development Goal, 2000), is to "stop the increase and start reversing the incidence of tuberculosis by 2015" [2].

To be able to assess whether these targets are met, it is important to continuously monitor the epidemiological situation of tuberculosis in the population. Annual risk of tuberculosis infection (ARTI) which is defined as the probability of acquiring new TB infection in the course of one year [3] is a sensitive indicator of the epidemiologic situation of tuberculosis [4]. It indicates the current and future magnitude of the incidence and prevalence of disease [5], and the average duration of infectious cases in the community [4]. It also expresses the efficiency of control activities. This indicator can be obtained from the prevalence of tuberculosis infection[6].

The most suitable method to determine the prevalence of infection with Mycobacterium Tuberculosis with reasonable likelihood is the tuberculin skin test (TST) [7]. There are various methods to estimate this prevalence from the results of TST surveys. In the simplest method, a cut-off point (COP) is used to estimate the presence of Mycobacterial infection. Any induration more than a preset cut-point is considered as infection. The value proposed for this cut-off may vary according to different considerations [8], but 10 mm is commonly use around the world including Iran. There are many challenges in the sensitivity and specificity of this method. Moreover, many informative data will be ignored (data reduction) while considering only one point to determine the infection [7].

Other methods, such as mirror and anti-mode methods have been recommended in many TST

surveys by World Health Organization. In these methods, criteria for estimating the prevalence of infection are determined from the frequency distribution of reaction sizes [6]. These methods, do not assign all individuals to infected or non-infected status[8] therefore the phenomenon of data reduction will not been occurred.

In the areas with a low prevalence of non-specific tuberculin reactivity, the frequency distribution of reaction sizes shows two distinct modes and a clearly identifiable anti-mode. Therefore, the anti-mode method (AM) is used to separate all participants in two subgroups. The reactions larger than the anti-mode represent the infected children with tubercle bacilli, while reactions belonging to non infected children or children infected with mycobacterium non tuberculosis, will locate in the left of the anti-mode. [6]. The infection prevalence may also been estimated using mirror image method (MI). In this technique, one should multiply the area under the right half of the distribution by two and add the number at the mode [9]. The rational of this technique is that the indurations caused by M.Tuberculosis infection are distributed normally around the second mode [6].

Several studies have been conducted using the above methods to estimate prevalence of M.Tuberculous infection and ARTI in the world. Results of these methods were in agreement in TST surveys in Indonesia and South Africa [8, 10]. On the other hand, different methods showed different results in Korea and Zambia [7, 8].

Iran is a developing country with a high BCG vaccination coverage. Although different studies in northern and western parts of this country have been conducted to estimate the prevalence of M.Tuberculosis infection and ARTI, all of them have used only COP=10mm method [11-13].

There are many challenges about the validity of the traditional approaches using cut-point method [7]. The dependency of the results of new methods of TB infection prevalence to the mode of reaction sizes due to the prevalence of atypical mycobacteriums and also special situation of these mycobacteriums in Iran makes it necessary to use all methods for estimating the TB infection prevalence in this country. There is not any study in Iran to compare the results of different techniques in this regard. In the current study, we aim to compare the results of different methods in estimating the M.Tuberculousis infection prevalence in school children aged 6-9 years.

Methods

This cross sectional study was a part of a TST survey carrying out in Sistan area in South-East of Iran (with the highest incidence risk of TB) [14, 15] in 2012-2013. In this survey, elementary school children aged 6-9 years were selected using stratified two stage cluster random sampling method. In this study, a sample of 1997 students studying in 22 schools (9 in urban and 13 in rural areas as primary sampling units) were selected. All students in the first, second and third levels were included in the study.

We trained five testers from the experienced health workers. They were selected out of 20 health workers who had field experience, After a 14 days training program only five ones were selected with the best performance.

Before the survey, all school authorities were invited and their consent was obtained after acquainting them with the purpose and safety of the study. A passive consent with the information about the survey was provided to the parents 2 or 3 days before the testing. Ethical clearance was obtained by ethical Review committee of Kerman University of Medical Sciences (ethical no: k/91/243).

On the testing days, our teams were attending in the selected schools; the first member of the team collected personal data such as gender, age, past medical history of students particularly BCG status using standard forms. Each student was examined for BCG vaccine scar, skin rashes and body temperature. Children aged less than 6 and more than 9 years, with a history of skin rash, with high fever, receiving anti-TB drugs and students whose parents did not give consent were excluded from the study.

All children were administered with 0.1 ml 5TU-PPD (5 tuberculin units of purified protein derivative; Razi institute, Tehran, Iran). Reading the test results were undertaken 72 hours after testing by an experienced reader. The maximum transverse diameter of the induration was measured in millimeter by a flexible transparent ruler.

In order to maximize the accuracy of the results, we re-checked about 30% of the completed forms during the reading days.

Students with reactions larger than 10mm were referred to the nearest health center in the specified dates for further evaluations for TB.

Statistical methods

A frequency table of reaction sizes in millimeter was made. We then presented the information as histograms where the X axis showed the indurations(mm) and Y axis indicated the percentage of persons with the specified induration size.

COP method

In this method, reactions of <5 mm were considered negative, 5—9 mm borderline, and all reactions of 10 mm or more were considered positive [1]. The percent frequency of positive reactions was considered as the prevalence of infection.

We also estimated the infection prevalence by varying the cut point by 1-2 mm on either side.

AM method

In this method, when there were two distinct modes in the distribution of the reaction sizes and also a relative clear anti-mode in the bottom of the distribution between two modes was visible, children with M. Tuberculous infection and those without were clearly demarcated at the anti-mode [16]. Therefore, all reactions to the right of the anti-mode, represented the infection with M.Tuberculousis and all reactions to the left of the anti-mode were attributed to uninfected children or infections due to non-specific Mycobacteriums [4].

MI method

In this method, the total prevalence of infection was estimated by doubling the proportion of reactions larger than the second mode and adding to the frequency at the second mode. The rationale of this approach is that tuberculin reactions due to infection with Mycobacterium Tuberculosis are distributed normally around the mode [6]. Therefore, it was possible to estimate only the overall situation of the infection prevalence instead of the individual aspect.

The prevalence of infection was estimated by adding the number of infected children in all schools, dividing this number by the total number of children analyzed and multiplying it with 100.

Moreover, the agreement between the results of cut point and anti-mode methods was estimated using kappa statistic. We could not assess the measures of agreement between MI method and the other methods, because this technique only estimates the overall prevalence, not the status of infection in individual level.

For the analysis, we considered schools as primary sampling units and all analyses were carried out using survey analysis. Mean of reaction sizes among categorical variables were compared using Mann-Whitney U test and the prevalence of infection among different subgroups were compared using Chi squared test.

We estimated the infection prevalence in vaccinated/unvaccinated children, boys/girls and children aged 6-9 years using COP method, AM method and MI method. Then we assessed the patterns of the infection prevalence within these subgroups and compared them with the expected pattern in the literatures. All analyses were performed using Stata V.11 software.

Results

Having excluded non-eligible students, finally we had full data of 1708 ones (85.5% of 1997 enrolled students); the main excluding factors were: age outlying (1.3%). The mean (standard deviation) of age of students was 7.1(0.8), and 47.8 percent were boys. Of them, 55.2% were selected from rural schools and 44.8% were from urban schools. Evidences of prior BCG vaccination was found in 1643 (96.2%) of the students.

No significant difference between the mean induration size in children with and without BCG scar and also among different age groups were found (p=0.8 and 0.3 respectively). We also found a significant difference between mean of test reactions between boys and girls (1.02 vs. 1.05, respectively, Mann whitney U test: p=0.01). Most of them (84.5%) had no reaction to the TST. figure 1 represents the frequency distribution of reaction sizes among all children and children with and without BCG scar.

COP method

Among the study children, 65 (3.8%, 95% CI: 2.9%, 4.8%) had induration sizes larger than 10mm. The prevalence of infection in children with and without BCG scar were 3.9 %(2.8,5.5) and 1.6%(0.2,11.6) respectively (p=0.4). These preva-

lences were estimated 4.5% and 3.1% respectively for girls and boys (p=0.4). In different age groups, infection prevalence in students aged 6, 7, 8 and 9 years, were 2.2%, 4.7%, 4.3% and 3.4% respectively (p=0.1). (Table 1). Prevalence estimates for cut-off points of 9, 8, 11, 12 mm were 4.4%(3.3%, 5.9%), 5.1%(4.1%, 6.4%), 3.5%(2.5%, 4.9%) and 3.2%(2.2%, 4.5%) respectively.

AM method

The anti-modes of the frequency distribution in all children, students with and without BCG scar were 12, 12 and 5 mm respectively. Therefore the prevalences of M. Tuberculosis infection in these subgroups were 3.2%(2.4%-4.1%), 3.2%(2.2-4.7) and 7.8%(2.1-24.7) respectively. Using this method, it was estimated 3.8% and 2.1% infection for girls and boys respectively (p=0.2). We also estimated the prevalence of infection in 6, 7 and 8 year old children as 1.9%, 9.4% and 4% respectively. The infection prevalence for 7 year age group significantly differed from that of the other age groups (p<0.0001). We failed to estimate these rates for older ages, because no visible anti-mode was observed.

MI method

Using 14 mm second mode of reaction size distribution in all children, the prevalence of infection was 5.1%(4.1%-6.4%). We also estimated the prevalence of infection for children with and without BCG scar as 5.2%(second mode=14mm) and 10.9% (second mode=6mm) respectively (p=0.2). The corresponding figures for girls and boys were 6.1%and 3% respectively (p=0.03) and for the above age groups were 1.9%, 13.3% and 3.8% respectively (p<0.0001). The infection prevalence for higher age groups was not estimated due to unobservable second mode in the distribution of reaction sizes in these age groups.

Measures of agreement

The kappa between COP and AM techniques was 90%; however, among those who had BCG scar, it was as low as 31%. In all ages and also in boys and girls the subgroup kappas were above 80%, except in 8 years old which was 65% (table 2)

Significant difference between subgroups

Table 2. The agreement between the results of cut-off point 10mm and anti-mode method in different subgroups

	Positive percent agreement	Kappa*
All children	3.16	0.91
With BCG scar	3.22	0.90
Without BCG scar	1.56	0.31
Males	2.08	0.80
females	3.82	0.91
6 years	1.86	0.91
7 years	4.79	0.65
8 years	3.98	0.96

**Kappa statistic in all subgroups was statistically significant (p<0.0001)*

Table 1. Results of different methods on the estimating the prevalence of tuberculosis infection in different subgroups

	The prevalence of infection (%)		
	Cut-off point=10mm	Anti-mode	Mirror image
All children	3.8(2.9-4.8)	3.2(2.4-4.1)	5.1(4.1-6.4)
BCG status			
with BCG scar	3.9(2.8-5.5)	3.2(2.2-4.7)	5.2(4.2-6.5)
without BCG scar	1.6(0.2-11.6)	7.8(2.1-24.7)	10.9(3.3-30.7)
Gender			
girls	4.5(3.2-6.2)	3.8(2.7-5.3)	6.1(4.6-8)*
boys	3.1(1.7-5.4)	2.1(0.9-4.6)	3(1.7-5.4)
Age(years)			
6	2.2(1.4-3.6)	1.9(1-3.4)	1.9(1-3.4)
7	4.8(2.8-8.2)	9.4(7.3-12.1)*	13.3(10.3-16.9)*
8	4.3(2.9-6.4)	4(2.7-5.9)	3.8(2.5-5.7)
9	3.4(0.4-24.4)	Not estimated	Not estimated

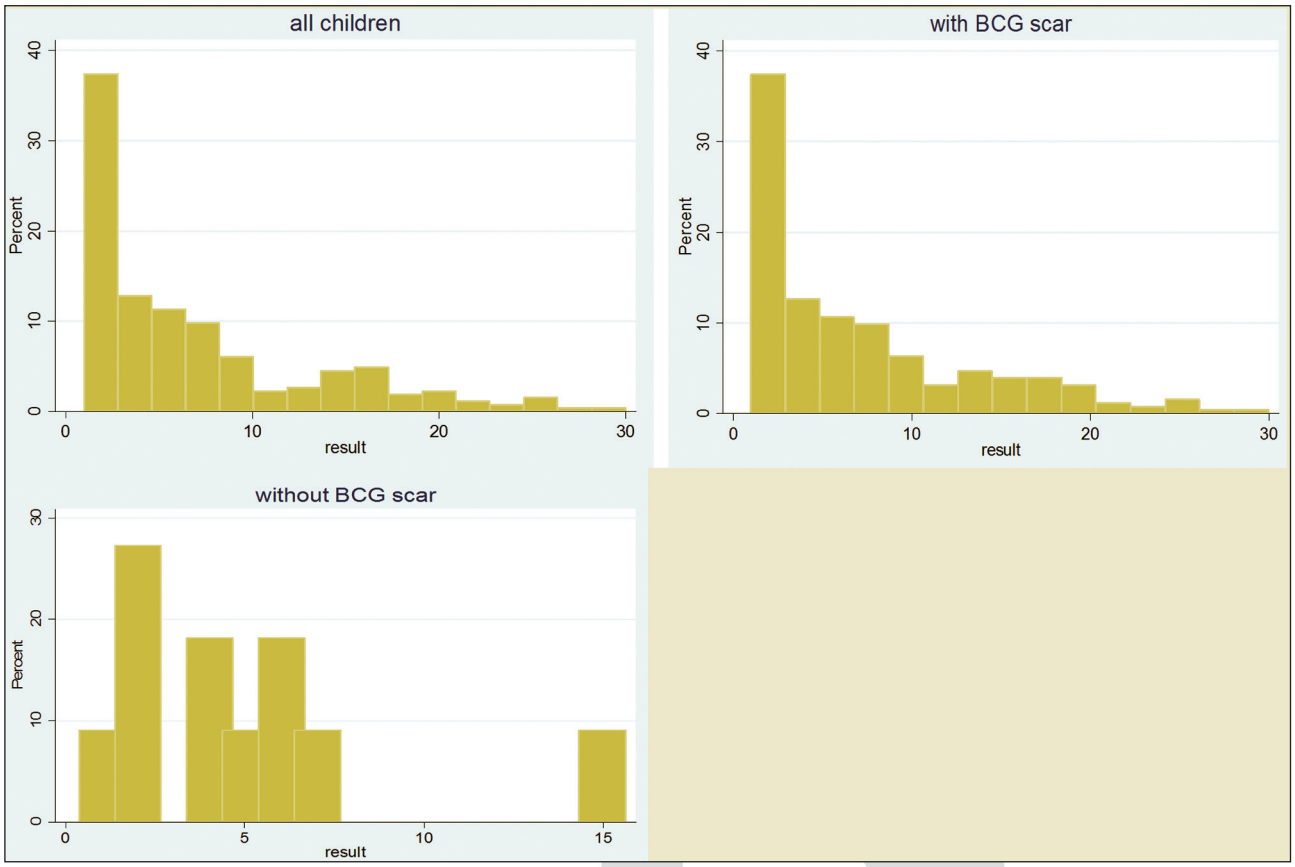

Figure 1. The frequency distribution of non zero reaction sizes among all children, classified by the presence or absence of the BCG scar

Discussion

We found in this study, which was the first TST survey in south-east of Iran, that estimates of infection prevalence using the traditional COP 10mm method was differed from those obtained by more suggested methods particularly MI method; and had relatively agreements with the results of AM method. Results of the MI method, were in agreement only with those of cut-off point=8 mm. We also detected significantly different TST results between genders and age groups when using AM and MI techniques.

Estimation of M.Tuberculosis infection prevalence among school children using COP=10 mm, AM method and MI method, irrespective of their BCG scar status showed different estimates. The largest prevalence was estimated by MI method and the lowest prevalence was calculated using AM method.

Similar to our findings, in a TST survey conducted in Zambia, the MI method showed higher estimates of infection prevalence[8] but results of TST surveys in Korea [7] and India [3] reported higher prevalences estimated by COP=10mm compared to the other methods. Conversely, In south African TST survey, similar results among all the above methods were found [8].

When we used different cut-off points, we estimated various infection prevalences. Results of COP=8mm yielded closest estimates to those obtained by the MI method. Similar situation was observed in the Korean TST survey which the COP=9 mm estimated better calibrated prevalence estimates [7].

Results of most studies reported no statistically significant differences in TST results among children with and without BCG scar [3, 11, 13, 17]. In our study, results obtained by all three methods were in agreement with these studies, while this pattern was in contrast to that of Indonesian survey[4] and survey conducted in Djibouti[18] reported higher rates of prevalence in BCG scar positive children than those without BCG scar. However we found nonsignificant differences in two groups and it should be noted that a 1–3%

difference in the infection prevalence estimates among children with a BCG scar and those without, may be significant in countries with a low prevalence of tuberculous infection [8].

Results of AM and MI method in our study showed significant higher estimates in girls than in boys. That was in agreement with the WHO reports of the situation of TB in children and adolescents in all regions including Iran [19], but were in contrast to the results of the TST surveys in India, Korea and Southwest of Iran [3, 7, 11].

Many studies reported significant effect of age on the tuberculin results [7, 8]. In the current study, the estimated prevalence of infection obtained by MI method and AM method was significantly associated with age, although the relevant pattern differed from that indicated in the literature. In our study, none of these methods found a linear trend in estimated prevalence with increasing age as expected in the previous surveys. It should be noted that persons with higher ages have been exposed more than those with lower ages to the M.Tuberculosis.

According to the above estimates of TB infection prevalence in different subgroups, it seems that the pattern of the TB infection in genders estimated by the MI method was closer to the real situation of the disease in the world.

In our study, we observed very good agreements between the prevalences estimated by COP=10mm and AM methods in all children and in children within different subgroups. Only the agreement in students without BCG scar was poor which could be due to the most difference between the anti-mode of the reaction distribution and the COP=10mm in these children. This might be a signal of influence of BCG vaccination on the TST results at least in Iranian children. Findings of the TST survey in Indonesia showed that the estimated ARTI based on the AM method was in agreement with that estimated using MI method only in subjects without BCG scar[4], while in Korea, using COP=10mm showed a closer concordance with the results estimated by more sophisticated statistical methods(mixture model analysis[7].

There are many challenges about the validity of COP method. Cut-off based methods may lead to biased and uncertainty estimates. Since this method does not provide information with regard to sensitivity and specificity of the test criterion, these are necessary to be specified from other sources. In this method, only the cut-off value is taken into account and all values on the either side of this point will be ignored. Most of the problems arise from this data reduction, whereas the original data may be much more informative[7]. Moreover, the specificity and sensitivity of tuberculin skin test is different in various geographical areas[20].

When the TST survey is used for epidemiological purposes to estimate the prevalence and risk of tuberculous infection, it is preferable to base the estimate not on the criterion that might be recommended for clinical practice in individual management, but on a direct analysis of the way in which the reactions are distributed according to size [16].

The MI method was used to determine the number of infected persons in the selected groups when detailed information about the induration size distributions were available. In this method, we assumed that nonspecific reactions to tuberculin are only infrequently larger than the mode of the distribution of the M.Tuberculosis induration sizes. Therefore, the distribution on the right side of the observed mode of true reaction is not contaminated by the nonspecific reactions[5]. The rationale is that the influence of BCG induced tuberculin sensitivity on the estimate of infection prevalence is neutralized if the estimation is done using the MI method. On the other hand, results of the MI method depend heavily on the mode values. For example, in a TST survey conducted in Djibouti, different values selected for mode, yielded different estimates of infection prevalence, while very similar results at different COPs of reaction sizes were obtained[18]. In addition, a clear anti-mode is not always detected, especially in communities with high prevalence of cross-sensitivity to tuberculin[8]. Moreover, we cannot estimate individual aspect of the infection using this method.

In this study, the results of COP 10mm method were lower than those of the MI method. This difference represented a lower sensitivity of the COP method than MI method. Therefore many indurations that were because of Mycobacterium Tuberculosis, were considered as environmental mycobacteriums when using COP=10mm method. Thus, the latter method suggested higher prevalence of non-tuberculous mycobacteriums than MI method. Moreover, the frequency distribution of the test re-

actions in this study showed a clearly bimodal distribution with an easily identifiable anti-mode. This kind of distribution is observed in areas with a low prevalence of non-specific tuberculin reactivity [6] such as the study area [21]. Thus, among the above methods, the mirror image method showed better agreement with the low prevalence of mycobacterium other than tuberculosis which is in keeping with the current situation of the study area.

In our study, the sample size of unvaccinated children was not enough for precise estimation of infection prevalence among unvaccinated children due to the high BCG vaccination coverage in Iran.

Failure to test and particularly read the results of all registered children might be a source of bias in our study due to the fact that those were absent in the testing and reading days were in different risk of TB infection. However, we tried to find and measure the reaction sizes of all absent children as much as possible.

Unfortunately, we did not have a gold standard method to compare the results of different techniques and find the most precise method.

In conclusion, our study and several related articles provided clear evidences that the traditional COP=10mm method does not have enough agreement with more accurate methods in estimating the TB infection prevalence. These estimates should be obtained by more sophisticated methods or at least using cut-off points which are in agreement with the MI methods.

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Evaluation of internal jugular and vertebral vein pathologies in multiple sclerosis cases using color doppler and B-mode sonography

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Abstract

Background and Aim: Multiple Sclerosis (MS) is a chronic demyelinating and degenerative disease that affects the central nervous system. Chronic Cerebrospinal Venous Insufficiency (CCSVI) is a recently described condition that may possibly contribute to the symptoms often experienced by patients with MS. We aim to compare the hemodynamics and the morphology of the internal jugular veins and vertebral veins using Color-Doppler and B-mode sonongraphy in MS cases and in healthy individuals as a control group. Also, flow volume of internal jugular vein (IJV) and vertebral vein (VV) in both supine and sitting position in MS and control groups.

Materials and Methods: IJV and VV of 60 MS patients and healthy individuals were examined using Color-Doppler and B-mode ultrasound in sitting and supine positions. Changes in hemodynamics and the presence or absence of morphological changes were recorded and evaluated according to Zamboni criteria. The presence of at least two of the Zamboni criteria in the same individual was considerede positive for evidence of CCSVI. Presence of the extracranial Zamboni criteria and measurements of flow volume were examined on supine and sitting positions for both group.

Results: In this study all the patients and control subjects were evaluated according to the first 4 criteria but the last criteria (reflux in the deep cerebral veins) was not assessed due to the lack of the required ultrasound probe in our institution. A subject was considered CCSVI positive if at least 2 Zamboni criteria were fulfilled. According to the described criteria, 76% of the MS patients showed abnormal findings and 21% of them showed evidence of CCSVI, however; only 33 individuals in control group showed abnormal findings and 1.6% of them showed evidence of CCSVI. The difference between groups is statistically significant ($p<0.01$). In addition we did not detect any statistically significant difference between both group's IJV-VV mean flow volumes which were examined in both sitting and supine positions ($p>0.01$).

Conclusion: There is a strong association between CCSVI and MS. This relationship can be easily demonstrated with B-Mode and Color-Doppler Sonography: a simple and non-invasive method. However further large studies and meta-analyses are required for the accurate determination of this association.

Key words: Multiple Sclerosis, Chronic Cerebrospinal Venous Insufficiency, Color-Doppler Sonography, B-Mode Sonography, Zamboni criteria.

Introduction

Multiple sclerosis (MS) is a chronic, primarily inflammatory demyelinating and degenerative disease that affects the central nervous system (CNS)[1]. It is estimated that it currently affects approximately 35000 individuals in the Turkey[2]. It is a disease with disseminated brain and spinal cord lesions, a wide variety of different neurological symptoms, and typically a fluctuating clinical course[3]. The symptoms of this multicentric process are manifest during acute relapses of the disease or after a period of disease progression. While the clinical course of MS is highly variable, in most cases it leads to severe and irreversible disability[4]. Multiple factors such as; genetic, infectious, environmental, and nutritional, etc., have been implicated in the etiology of MS and an interaction between various factors could potentially explain the heterogeneity observed in studies of both the pathological character of MS lesions and the nature of disease manifestations[3,4].

Chronic cerebrospinal venous insufficiency (CCSVI) is a recently described condition that may possibly contribute to the symptoms often experienced by patients with MS[5]. The idea that the vascular system is somehow related to the pathological abnormalities of MS was proposed shortly after the characteristic lesions were described[6]. The venous stenoses described in CCSVI involve predominantly the internal jugular and azygos veins, but in theory because it is the existence of venous obstruction that is important, rather than its precise location, the narrowing of other veins draining the cerebrospinal axis may play a role[5-7]. These may include intracranial channels such as major intracerebral veins, ophthalmic veins, petrosal sinuses and durai sinuses, as well as extracranial vessels including the vertebral veins, left brachiocephalic vein or normally existing collateral neck veins in terms of venous insufficiency affecting the more commonly involved internal jugular and azygos veins, the presence of multiple lesions affecting one or more veins is frequently observed in CCSVI[5-8]. CCSVI can be easily diagnosed using Color-Doppler sonography by estimating the extracerebral venous hemodynamics which changes in posture, and to state the morphology of the venous system in MS patients[9,10].

The aim of this study is to compare the hemodynamics and the morphology of the internal jugular veins and vertebral veins using Color-Doppler and B-mode sonongraphy in MS cases and in healthy individuals as a control group. Also, flow volume of internal jugular and vertebral veins in both supine and sitting position in MS and control groups.

Materials and Methods

Subjects

This case-control study was approved by the Ethics Committee of Yuzuncu Yıl University and informed consent was obtained from all subjects. A total of 60 patients with clinically defined MS were assessed according to McDonald criteria[5,6]. The patients (42 women and 28 men) were aged 20-60 years, with a median age of 35.7±14.8 years. They had suffered from MS for 4 to 23 years (median disease duration; 14 years). The clinical type of MS could be determined in 60 cases; 52 patients (86.67 %) had the relapsing-remitting, 4 patients (6.67 %) had the secondary progressive, and 4 patients (6.67 %) had the primary progressive course of MS. The control group consisted of 60 neurologically normal individuals who were sex-matched and age-matched with the MS patients.

Exclusion criteria

Exclusion criteria were: presence of relapse and steroid treatment in the 30 days preceding study entry for all patients, pre-existing medical conditions known to be associated with brain pathologies, such as neurodegenerative disorders, alcohol abuse, and other conditions, contraindication for having a contrast agent injected for MRI examination, history of cerebral congenital vascular malformations or pregnancy.

Study Protocol

IJV and VV of 60 MS patients and healthy individuals were examined using Color-Doppler and B-mode ultrasound in sitting and supine positions. The Doppler exam was performed using Philips HD-11 Ultrasound machine (Bothell, Washigton, USA) equipped with 2.5 and 7.5-10 MHz transducers and motorized chair capable of tilting from 0° to 90°. All study examinations were performed by the same Doppler technologist. All subjects were examined first in the supine and then in the sitting position (0° and 90° respectively) in accordance with the previously reported CCSVI protocol.

Assessment of Doppler flowmetry in mL/min by using the softwares included in the package of the ultrasound equipment was performed in the middle part of bilateral jugular vein for the internal jugular vein at the level of C5-C6 for vertebral vein.

Excessive attention was made for the presence of the following parameters extra-cranial Zamboni criteria[5,6,11,12]; 1) Reflux/bidirectional flow in the IJVs and/or in the vertebral veins (VVs) in sitting and in supine positions (90° and 0°), defined as flow directed toward the brain for a duration of >0.88 s; 2) B-mode abnormalities or stenoses in IJVs, defined as cross-sectional areas (CSAs) of this vein ≤0.3 cm^2 with flaps, webs, septa, etc., in the lumen of IJVs, considered to be B-mode abnormalities significantly disturbing cerebral venous outflow; 3) Flow that is not Doppler-detectable in IJVs and/or VVs despite multiple deep breaths;

4) Reverted postural control of the main cerebral venous outflow pathway determined by measuring the difference between the CSA of the IJVs in the supine and in the upright positions. In this study all the patients and control subjects were evaluated according to the first 4 criteria but the last criteria (reflux in the deep cerebral veins) was not assessed due to the lack of the required ultrasound probe in our institution. A subject was considered CCSVI positive if at least 2 Zamboni criteria were fulfilled[5,6].

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 17.0). For statistics between the groups, the t-test, chi-square test and Mann-Whitney rank sum U-test were used. Prevalence rates for each of the five criteria, as well as for different CCSVI status groups, were calculated using the chi-square test. Reproducibility was calculated using Cohen's Kappa and inter-class correlation (ICC) tests. Values were calculated separately for MS patients and healthy controls. In order to avoid too many spurious findings due to multiple comparisons, a nominal p-value <0.01 was considered as significant using two-tailed tests.

Results

The mean age of the MS patients was 35.7±14.8 (min. 20-max. 60). There were no age or sex differences between MS patients and HC. CCSVI, defined as the presence of at least two positive venous hemodynamic criteria, was present in 20 of the 60 MS patients (33.3 %) and in 1 of the 60 controls (1.6 %) (p<0.01). Abnormal hemodynamic findings among the different groups are listed in Table 1.

The presence of at least 2 positive DS VH criteria indicative of CCSVI showed acceptable sensitivity (33.3%), specificity (98.3 %), negative predictive value (59.6 %) and positive predictive value (95.24 %) for MS and HC groups.

Presence of at least one positive venous hemodynamic criteria, was present in 46 of the 60 MS patients (76.67 %) and in 13 of the 60 controls (21.67 %) (p<0.01). Moreover, the presence of at least three positive venous hemodynamic criteria was present in 1 of the 60 MS patients (76.67 %) and none among the control group. Color-Doppler Sonography images recording from MS patient having three positive venous hemodynamic criteria together have shown in Figure 1, 2 and 3. Figure 1 shows echogenic filling defect in left IJV on supine position, Figure 2 shows reflux flow using valsalva maneuver in left IJV on sitting position and Figure 3 shows flow absence in left VV on supine position. Four positive venous hemodynamic criteria weren't detected both in MS and control groups.

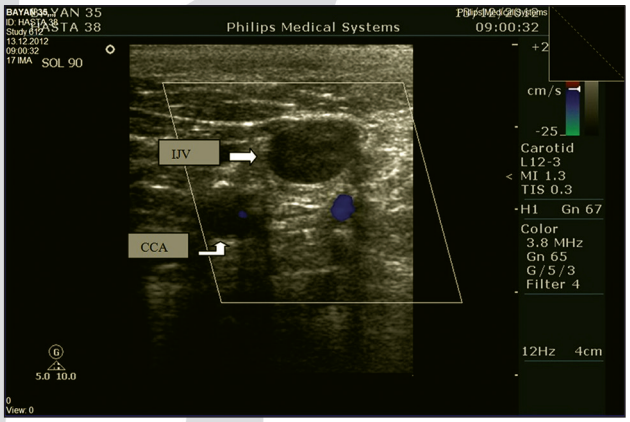

Figure 1. Echogenic filling defect in left IJV on supine position

Table 1. Abnormal hemodynamic findings among the different groups

	Control Group (n)	MS patients (n)	p value
Reflux/bidirectional flow in the IJVs and/or in the VVs in sitting and in supine positions:	5 (8.3 %)	20 (33.3 %)	p<0.01
High-resolution B-mode evidence of proximal IJV and/or VVs morphological abnormalities and stenosis	3 (5%)	22 (36.6 %)	p<0.01
Flow that is not Doppler-detectable in IJVs and/or VVs despite multiple deep breaths	5 (8.3 %)	18 (30 %)	p<0.01
Reverted postural control of the main cerebral venous outflow pathway	1 (1.6 %)	20 (33.3 %)	p<0.01

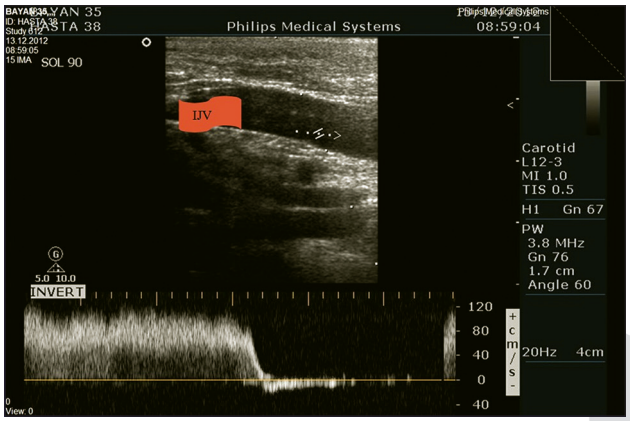

Figure 2. Reflux flow using valsalva maneuver in left IJV on sitting position

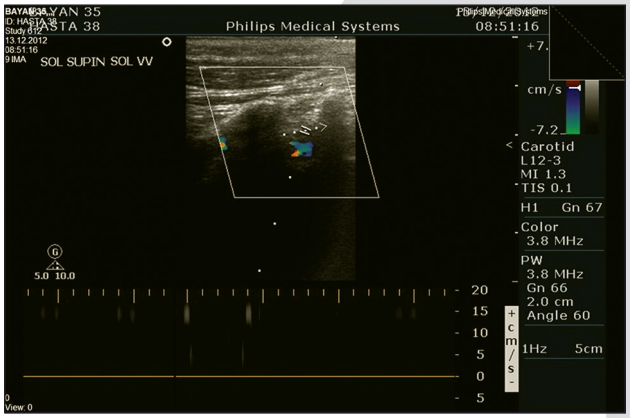

Figure 3. Flow absence in left VV on supine posit

Figure 4 shows presence of two echogenic septa in right IJV vein near the level of the existing opening in a female patient at the age of 27. Figure 5 and 6 show presence of reflux flow in left IJV and echogenic septa in right IJV in a male patient at the age of 35, respectively.

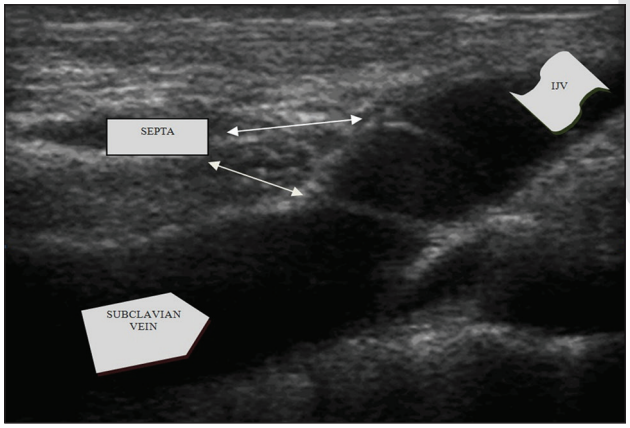

Figure 4. Presence of two echogenic septa in right IJV vein near the level of the existing opening

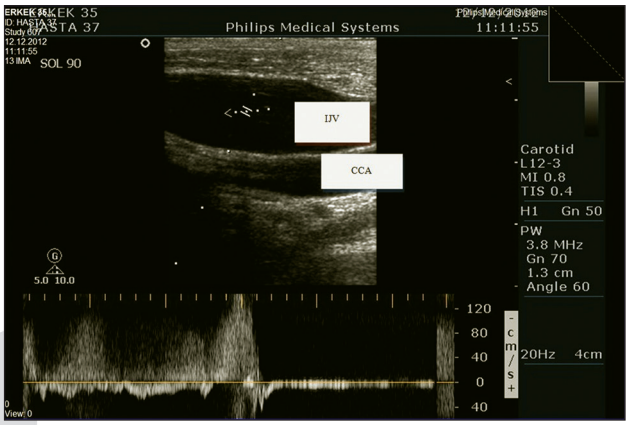

Figure 5. Presence of reflux flow in left IJV

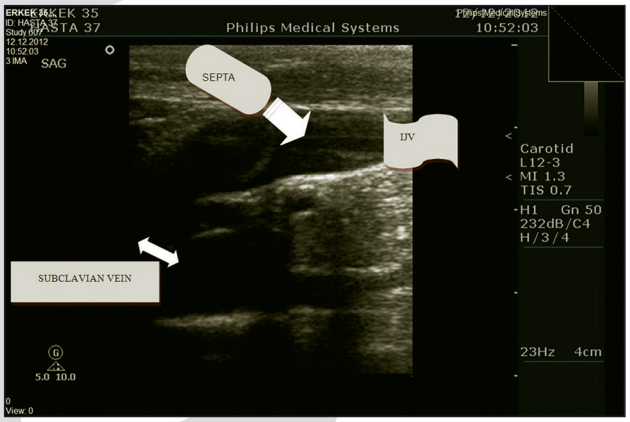

Figure 6. Presence of echogenic septa in right IJV

Although, most of the patients had relapsing-remitting type of MS, a few of the patients had the primary or secondary progressive course of MS. For this reason, subtype of MS cases couldn't be compared statistically with each other.

Mean flow volume of 60 patients were 537.35±228.46 [min. 143.0-max. 1153.0] mL/min on supine position and 302.25±218.36 [min. 48.0-max. 1149.0] mL/min on 90° in IJVs, 18±14.68 [min. 0.0-max. 68.0] mL/min on supine position and 82.48± 55.05 [min. 0.0-max. 261.0] mL/min on 90° in VVs. Mean flow volume of 60 healthy individuals were 547.90±297.65 [min. 167.0-max. 1357.0] mL/min on supine position and 238.93±186.26 [min. 55.0-max. 1024.0] mL/min on 90° in IJVs, 23.0±15.33 [min. 4.0-max. 99.0] mL/min on supine position and 88.53± 54.29 [min. 1.0-max. 270.0] mL/min on 90° in VVs.

Mean flow volume of patients having at least two positive extracranial Zamboni criteria (n=40) were 547.4±291.02 mL/min on supine position and 292.95±189.27 mL/min on 90° in IJVs, 20.35±20.73 mL/min on supine position and 83.05±57.36 mL/

min on 90° in VVs. Mean flow volume of patients not having at least two positive extracranial Zamboni criteria (n=20) were 532.33±193.97 mL/min on supine position and 306.9±233.7 mL/min on 90° in IJVs, 17.53±10.54 mL/min on supine position and 82.2±54.6 mL/min on 90° in VVs.

Mean flow volume of healthy individuals having at least two positive extracranial Zamboni criteria (n=1) were 540.0±287.47 mL/min on supine position and 213.0±103.829 mL/min on 90° in IJVs, 19.0±10.27 mL/min on supine position and 83.0±53.12 mL/min on 90° in VVs. Mean flow volume of healthy individuals not having at least two positive extracranial Zamboni criteria (n=59) were 551.75±306.135 mL/min on supine position and 251.425±216.22 mL/min on 90° in IJVs, 24.60±17.21 mL/min on supine position and 91.13±55.35 mL/min on 90° in VVs.

Discussion

MS is generally considered to be an immunomediated inflammatory disorder of the central nervous system influenced by environmental and genetic factors[1,2]. Zamboni and coworkers[5,6] recently described an association between MS and a condition defined as CCSVI, which is characterised by a high incidence of reflux in both intracranial and extracranial venous segments, loss of postural venous outflow regulation, the presence of multiple stenoses of unknown origin in the internal jugular veins and azygos vein, and the opening of collateral circles. This concept has had an impressive effect on the MS community[5,6,11].

In the original description of the association, combined Extracranial High-resolution Echo-color Doppler (ECD) and Transcranial Color-coded Doppler Sonography (TCDS) were used as non-invasive methods to detect CCSVI in 65 patients with MS and 235 control individuals. The team focused in particular on the detection of five abnormal venous hemodynamic parameters. The CCSVI diagnosis needed to fulfill at least two of these five parameters. The detection of two or more parameters in the same patient was not found in any of the healthy controls or patients with other neurological disorders, but perfectly overlapped with the diagnosis of clinically definite MS, in terms of a reported 100% sensitivity, specificity, positive predictive value, and negative predictive value. The ECD-TCDS findings in this study were verified by transfemoral selective venography, which also showed a significantly higher pressure gradient across the venous stenoses[6].

Zamboni et al.[12] designed a study with 109 MS subjects and 177 controls including both healthy subjects and other neurological diseases. According to this study, CCSVI criteria were found in all MS patients and in none of the control subjects (sensitivity 100%, sensibility 100%, positive predictive value 100, and negative predictive value 100%).

Simka and colleagues[13] assessed the extracranial Doppler sonographic criteria for CCSVI in 70 patients with different clinical forms of MS. Signs of abnormal venous outflow were noted in 64 (91%) patients with MS and 63 (90%) fulfilled the criteria for CCSVI. The most common pathological sign was the presence of an inverted valve or another pathological structure, such as a membranous or netlike septum, in the junction between the internal jugular and brachiocephalic veins.

Al-Omari and Rousan[14] reported that 23 of 25 (92%) patients with MS and six of 25 (24%) control individuals had abnormal extracranial internal jugular vein findings. CCSVI criteria were met in 21 (84%) patients with MS and none of the control individuals. Furthermore, a recent transcranial and extracranial ECD study by Zivadinov and colleagues[15] also showed a significantly increased prevalence of CCSVI in patients with MS (62.5%) compared with healthy control individuals (25.5%) but with substantially lower sensitivity and specificity rates than originally reported.

According to Zivadinov et al.[15], 289 MS patients, 163 healthy controls and 26 subjects with other neurological diseases were evaluated with the same approach described by Zamboni et al.[12], finding the pattern of CCSVI, respectively, in 56%, 22.7% and 42.3% of cases. Venous abnormalities were correlated to the severity of brain damage, measures of brain atrophy, and to the severity of motor, cerebellar and brainstem involvement.

Doepp et al.[16] studied 56 MS patients and 20 healthy subjects with extra- and transcranial color-coded sonography, finding no venous stenosis in any of them; none of the subjects investigated in this study fulfilled criterion for CCSVI leading to the conclusion that cerebral venous congestion is

unlikely to play a significant role in the pathogenesis of MS.

Laupacis and colleagues[17] designed a meta-analysis for standardization to arise the different results due to the geographical distribution of different age groups and different genders between CCSVI and MS groups. According to this meta-analysis, CCSVI was detected in 352 of 632 MS patients and 58 of 510 healthy individuals (OR=13.5, 95% CI=2.6-71.4).

In our study, presence of at least one extracranial Zamboni criteria, was present in 46 of the 60 MS patients (76.67 %) and in 13 of the 60 controls (21.67 %) ($p<0.01$). According to our study, the most common detected single criteria in MS patients was evidence of proximal IJV and/or VVs morphological abnormalities and stenosis (22/60), in the second rate it was reflux/bidirectional flow in the IJVs and/or in the VVs in sitting and in supine positions (20/60). Simka and et al.[13] and Al-Omari and Rousan[14] found similar findings in their study.

Our study is consistent with the overall study of the Laupacis and colleagues[17]. Although , the most rarely seen single criteria in MS patients was negativity of CSA in our study, according to Laupacis et al.[17], it was evidence of proximal IJV and/or VVs morphological abnormalities and stenosis. On the other hand, Simka et al.[13] found that the most rarely seen single criteria were negativity of CSA, similar to our study.

In our study, unilateral flow was not detected in 17 patients and bilateral flow was not detected in 2 patients. Among these 17 patients, 9 of them had left vertebral vein pathology and 8 of them had right vertebral vein pathology. There was no significant difference between the affected sides. In control group, vertebral vein flow was not detected in three individuals. Two of them were right side pathology; one of them was left side pathology. None of them had bilateral flow pathology.

All of the patients having two or more Zamboni criteria had IJV pathology and 12 of them had VV pathology. In control group one individual having two Zamboni criteria had normal VV morphology, carotid ectasia compressing to IJV in the right side and lack of IJV flow in the left side.

The greatest limitation of this study was lack of appropriate probe, for this reason we couldn't determine reflux flow in cerebral vein. In the absence of this restriction, we predicted that CCSVI rate in MS patients and healthy individuals would be a little higher than the current situation.

Zamboni et al.[12] found that mean flow in VV was 40±20 mL/min and 210±120 mL/min in supine and sitting positions, respectively. In our study mean flow in VV was 23±15.33 mL/min and 88.53±54.29 mL/min on supine and sitting positions, respectively. We have noticed that our mean values were lower than Zamboni et al.[12], we thought that the reason of this was making the studies in different geographies.

According to Zamboni et al.[12], mean IJV flow values were found as 700±270 mL/min and 70±100 mL/min on supine and sitting positions, respectively. In our study mean flow in VV was 547.9±297±65 mL/min and 238.93±186.26 mL/min on supine and sitting positions, respectively. There were no significant differences between our study and Zamboni and colleagues' study for control and MS groups.

Conclusion

It is obvious that, we still know far less than is necessary to other patients and their families' durable symptom relief, much less a cure for MS. All interested parties continue to be confronted with more questions than answers regarding the pathogenetic basis of MS, maintenance of the disease, subtypes of disease expression, and most effective and predictable therapeutic strategies[18-21]. Historically, various aspects of vascular dysfunction have been described in MS[22]. Epidemiological studies suggest that patients with MS have a higher risk of ischemia stroke than individuals who do not have MS and multiple diagnostic modalities have demonstrated that patients with MS exhibit global cerebral hypoperfusion[23].

CCSVI is a new hypothesis that has captured the front page interest of patients worldwide and although large, multi-institution trials suggest it has an association with MS, the evidence to confidently support its fundamental role in the pathogenesis and progression of this disease is currently lacking[24-29]. This relationship between CCSVI and MS can be easily demonstrated with B-Mode and Color-Doppler Sonography: a simple and non-invasive method[30-32].

It is readily appreciated that MS is a complex pathological process, not unlike certain cancers, with its etiology influenced by a variety of factors (genetic, infectious, environmental, dietary, inflammatory, etc.)[33-35].The precise interplay and requisite role(s) of contributing factors that lead to the pathogenesis of MS is unknown[1,4,7]. It is possible that the mosaic combination of individual etiological elements responsible for disease expression is different and even unique among those affected[1,4,7,8]. It is currently unknown whether CCSVI represents one of the factors or risk factors that promotes or enables-along with others-the development of MS[36-38].

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Gene polymorphism studies in glucose 6 phosphate deficiencies in Saudi Arabia

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme in the pentose phosphate pathway (PPP) that plays an important role in protecting cells from oxidative damage by producing NADPH and reduced glutathione. G6PD deficiency is considered one of the most common genetic disorders present in the X chromosome and is the most common of enzymopathic red blood cell disorder. We evaluated the role of three single nucleotide polymorphisms (SNPs), factor V Leiden G1691A (FVL), Prothrombin gene mutation G20210A (PRT or FII-G20210A) and methylenetetrahydrofolate reductase variant C677T (MTHFRC677T), as risk factors for G6PD in Saudi populations. Our results did not show any association with the three Thrombophilic genes with FVL gene, no statistical analysis have shown any association with either allele or genotype frequencies ACE I/D polymorphism study was carried out in G6PD individuals and showed strong association with DD genotypes and D alleles OR=39.38, p<0.0001 (95% CI=8.80-176.1) and OR=38.58, p<0.0001 (95% CI=13.21-112.6). Another gene of α2BAR I/D polymorphism study cannot show any association in DD genotype and with D. Our study shows that G6PD deficiency is showing strong association in DD genotype and D allele of ACE gene and rest of the genes have not shown any important role and one of the reason could be the low sample size.

Key words: G6PD, MTHFR, FVL, PRT, ACE, Saudi population.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is a metabolic enzyme that catalyses the first reaction in the pentose phosphate pathway (PPP), providing reducing power to all cells in the form of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate). Erythrocytes do not generate NADPH in any other way than PPP, they are susceptible to oxidative damages and by this way NADPH enables cells to counter balance oxidative stress triggered by oxidant agents. G6PD enzyme is critically important for protecting erythrocytes from oxidative stress and intravascular hemolysis. G6PD is an X-linked gene encoding the first enzyme of the PPP, a NADPH-producing dehydrogenase [1].

G6PD deficiency is considered one of the most common genetic disorders and is the most common of enzymopathic red blood cell disorder. Worldwide 400 G6PD deficiency individuals were asymptomatic but acute hemolysis may occur in certain conditions. It may cause chronic nonspherocytic hemolytic anemia. The common clinical manifestations of G6PD deficiency are jaundice and acute hemolytic anemia triggered by certain drugs, infections or ingestion of fava beans**.** The disease has been reported in populations from nearly all geographical locations; however it occurs most frequently in areas where Plasmodium falciparum malaria has been endemic. Prevalence estimates are highest in Africa, Asia, the Mediterranean region and the Middle East. The G6PD gene maps to the tip of the q arm of the X chromosome (Xq28), and consists of 13 exons encoding a 515 amino acid protein with a molecular weight of about 59 kDa [3]. The World Health Organization has grouped the G6PD variants into five disease classes based on enzymatic activity levels; however the clinical impact of even some of the endemic variants is not completely defined [4].

In the present study we have selected 5 genes among them two are Insertion/ Deletion genes (Angiotensin-Converting Enzyme (ACE) and α_{2B}-adrenergic receptor gene (α_{2B}AR) and remaining three were thrombophilic genes Factor V Leiden (FVL), Prothrombin (PRT) and (methylenetetra-

hydrofolate reductase (MTHFR). The importance of the Prothrombin genes are the mutation represents one of the most important risk factors for inherited thrombophilia.

The ACE gene contains a polymorphism based on the presence (insertion [I]) or absence (deletion [D]) within an intron of a 287-base-pair (Alu Sequence) nonsense DNA domain, resulting in three genotypes (DD and II homozygotes, and ID heterozygotes). ACE is an important enzyme of the renin-angiotensin-system, which is encoded by the ACE gene. This enzyme catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, and also inactivates the vasodilator bradykinin. The α٢BAR insertion/deletion (I/D) polymorphism involves the deletion of three glutamic acids from a repeat element in the third intracellular loop of the protein. In human studies, α٢BAR I/D polymorphism were found to be associated with various cardiovascular and metabolic phenotypes. A common variant (12Glu9) of the human α٢BAR gene encodes a receptor protein leading to the I/D of three consecutive glutamate residues at amino acid positions 301 to 303 has been associated with EHT, acute coronary events. In-vitro studies showed, I/D polymorphism of α٢BAR gene to affect an impaired agonist-induced desensitization of the receptor [5].

FVL is a point mutation which impairs the proteolytic degradation of the factor V by protein C that makes it active for a long time. This abnormality of the hemostasis system is associated with an increased risk of venous thrombosis. The prevalence of the mutation varies among different populations, being high in Europeans and almost absent in the populations of Southeast Asia, Polynesia and Africa. A nucleotide transition at G20210A in the 3'untranslated region of the prothrombin (PRT or FII) gene was associated with nearly a 25% increase in plasma thrombin activity and a 2.7-fold increased risk for venous thrombosis. G20210A is associated with increased plasma levels of PRT. It is the 2nd most common inherited risk factor for thrombosis**.** The FVL (G1691A) and PRT (G20210A) mutations are 2 such examples; each has been found to be associated strongly with spontaneous and recurrent venous thromboembolism. Similarly, a thermolabile variant of MTHFR is an enzyme involved in the folate-dependent metabolism of homocysteine, increases the risk for deep vein thrombosis and pulmonary embolism. MTHFR is a key enzyme in folate and homocysteine (Hcy) metabolism. A single point mutation causing a C-T substitution at nucleotide 677 and from the valine to alanine in the enzyme of the MTHFR gene has been associated with a thermolabile enzyme form of low biological activity. C677T MTHFR polymorphism is associated with low folate and vitamin B12 levels.

G6PD is X-linked and so deficient variants are expressed more commonly in males than in females. The evolutionary conservation of a housekeeping gene such as G6PD is greater than that of tissue-specific genes, presumably because the latter may require more specific adaptation to the physiology of individual organisms. Total lack of G6PD is almost certainly lethal in mammals, but partial G6PD deficiency confers biological advantage with respect to malaria. Most of the replacements causing G6PD deficiency take place in positions that are conserved, but less than fully conserved. Thus, the range of permissible G6PD mutations in the microevolution of the human species bears a clear imprint of the macro evolutionary history of this gene. With increasing numbers of full genome sequences becoming available, the approach we have used for the analysis of G6PD mutations is likely to be generally applicable to genes (particularly housekeeping genes) underlying other human diseases [9]. The abundance of distinct mutation sites and their clinical manifestations make this enzyme ideal for structure-function analysis studies. The main goal of the present study is to screen of G6PD deficiency in the blood donors in Kingdom of Saudi Arabia and also in this analysis, we also summarise the occurrence or degree of mutations by residue conservation of G6PD sequence in various species.

The aim of the present study was to assess the role of selected genes does play any important protagonist in the G6PD individuals in Saudi Arabian men and the other goal of this study is to screen of G6PD deficiency in the blood donors in Kingdom of Saudi Arabia and also in this analysis, we summarises the occurrence or degree of mutations by residue conservation of G6PD sequence in various species.

Materials and Methods

Selection of G6PD Individuals

In this study 61 samples were included and among them 22 samples were healthy controls without any complications. This preliminary study included 39 G6PD male individuals who have visited to Central blood bank and driver school blood bank (DALLA), King Abdul-Aziz specialist Hospital in Taif city in West region of Kingdom of Saudi Arabia during the period 3rd July 2011 to 12th Aug 2011. The Age groups of these donors were 17 to 50 years with a mean age of 33.5±9.96. All the G6PD individuals were confirmed G6PD determination and quantitative test.

6 mL of EDTA Blood sample was collected from every male donor and 4 mL of the sample was used for hematological parameters to confirm G6PD deficiency and 2 mL of the blood was used for molecular analysis. Institutional ethical committee approval and informed consent was obtained from all the subjects included in the study. The inclusion criteria of G6PD were to include male donors and female donors were excluded from the study.

Analysis of G6PD variants

Genomic DNA was extracted from peripheral blood leukocytes using Norgen DNA extraction kit (Norgen Biotek corp, Canada). The procedure followed laid out norms for DNA extraction. The genotype analysis was performed at the Department of Genetics and clinical laboratory sciences, College of Applied Medical Sciences, King Saud University, Riyadh and Saudi Arabia.

Polymerase chain reaction (PCR) was carried out to determine Thrombophilia genotyping of 3 genes (FVL-PTR-MTHFR) was performed by using PCR-RFLP and the ACE and α_{2B}AR D/D, I/D, and I/I genotypes and specific Insertion/Deletion primers as shown in the table 1 were chosen from our earlier studies. Primers were synthesized by Bioserve technologies, Hyderabad, India).

A total volume of 20 µL reaction mixture contain 2 µL of each primer which is 100 pmole, 6 µL of sterile water and 10 µL of 2X master mix which includes $MgCl_2$, 10x Taq buffer, 10 unit of Taq DNA polymerase (Norgen Biotek corp, Canada) and the 2 µL template DNA was used for amplification of I/D polymorphisms of ACE gene and α_{2B}AR gene.

For the genotyping I/D genotype variant of the ACE gene was determined by PCR amplification. PCR products of 190 bp (without insertion) and 490 bp (with 287-bp Alu insertion) were detected by electrophoresis on agarose gel containing ethidium bromide. Amplification of the PCR products, which is a 490-bp fragment for the I allele and a 190-bp fragment for the D allele, was carried out using the following: The PCR profile consisted of 35 cycles (denaturized at 94 °C in 5 minute, annealing at 59 °C in 30 seconds and extension at 72 °C for 5 minute) was used.

The PCR cycling conditions were carried out on Thermal Cycler (Applied Biosystems, Hercules, California, USA). The 1st Exon of the polymorphic α_{2B}AR gene the initial denaturation was set up for 5 minutes at 95 °C followed by 35 cycles of denaturation for 30 seconds at 95 °C, annealing for 30 seconds at 57 °C, extension for 45 seconds at 72 °C and the final extension was at 72 °C for 7 minutes. The insertion band is considered as 112bp which represents the I allele and 103 bp is identified as deletion band which represented the D allele. Insertion/Deletion of the amplified PCR products were clearly separated on 2.5% agarose gel (Cambrex, East Rutherford, NJ, USA) which has been performed in Origins electrophoresis (Elchrom Scientific AG, Cham, Switzerland).

Statistical Analysis

All the statistical analysis was carried out by using SPSS (Chicago, IL, USA) software version 19.0 for Microsoft Windows®. Clinical characteristics of all the subjects were expressed as mean ± SD. Continuous variables were compared between the groups by using two-tailed student's *t* test. Allelic frequencies were calculated by gene-counting method and the genotypic and allelic distribution of I/D polymorphism of α_{2B}AR gene were analyzed using chi-square test. The effect of I/D genotypes of α_{2B}AR was analyzed using general linear model ANOVA for clinical characteristics. A level of $p<0.05$ was considered statistically significant.

Results

I/D polymorphisms were identified based on the band sizes of ACE and α_{2B}AR genes. Over all 39 men were participated were in this study and

women's were excluded from this study. G6PD individuals were in the age range of 17-50 years with a mean age of 33.5±9.96. It was seen that G6PD individual's blood groups were A, B and O of both positive and negative blood groups and none of the G6PD individual were not having both AB positive and negative blood groups. Table 1 shows the individuals participated in the study with their blood groups.

ACE I/D polymorphism study was carried out in G6PD patients were 82%, 7.7% and 10.2% respectively. The percentage of D allele was 85.9% and of the I allele was 14.1%.In the control subjects, the distribution of ACE DD, ID and II genotypes was 9%, 9% and 81.9% respectively. The allele frequencies was 13.6% and 86.3% for the D and I alleles, respectively. When we have compared the ACE DD genotypes and D allele with the cases and controls we have found a strong association OR=39.38, p<0.0001 (95% CI=8.80-176.1) and OR=38.58, p<0.0001 (95% CI=13.21-112.6). Similarly, the difference of D allele frequencies between patients and controls was found to be strong significant; 82% for patients and 9% for controls. Table 2 is showing the genotypes and allele frequencies of 2 different genes.

Table 2. G6PD Individual and percentages of their blood groups

Blood Group	Positive	Negative
A	10 (25.65%)	01 (2.56%)
B	04 (10.25%)	01 (2.56%)
AB	00 (0%)	00 (0%)
O	22 (56.41%)	01 (2.56%)

Another gene of α_{2B}AR I/D polymorphism study was carried out with G6PD group 4/39 (10.2%) cases showed DD genotype, 04/39 (10.2%) showed ID and 31/39 (79.5%) showed DD genotype. Among the cases 12 (15.4%) showed D allele and 66 (84.6%) showed I allele. In the control group 01/22 (4.5%) showed DD genotype, 05/22 (22.7%) showed ID genotype and 16/22 (72.7%) showed II genotype. In the control group 07 (15.4%) showed D allele and 37 (84.1%) showed I allele. A chi square test between the cases and control of D allele did not show a significance difference OR-0.9614, p=0.9388 (95% CI=0.3482-2.653) D Allele. None of the genotypes or allele did not show any association

Table 1. Genotype distribution of the polymorphisms examined in this study

Gene	Mutation	rs no	Forward Primer	Reverse primer	Fragment	Annealing Temp	Enzyme
ACE	INTRON 16	rs4646994	CTGGAGACCACTCCCATCCTTTCT	GATGTGGCCATCACATTCGTCACGAT	490 bp	59°C	-
α_{2B}AR	EXON 1	rs4426564	AGGGTGTTTGTGGGGCATCT	CAAGCTGAGGCCGGAGACACT	112 bp	57°C	-
FVL	G1691A	rs6020	TCAGGCAGGAACAACACCAT	GGTTACTTCAAGGACAAAATACCTG-TAAAGCT	241 bp	56°C	*HindIII*
PRT (FII)	G20210A	rs1799963	TCTAGAAACAGTTGCCTGGC	ATAGCACTGGGAGCATGAAGCAAGC	345 bp	60°C	*HindIII*
MTHFR	C677T	rs1801133	TGAAGGAGAAGGTGTCTGAGGGA	AGGACGGTGCGGTGAGAGTG	198 bp	68°C	*HinfI*

Table 3. Genotype and Allele frequencies of ACE, α_{2B}AR, FVL, PRT and MTHFR genes

Genotypes and Alleles	ACE Cases (n=39)	ACE Controls (n=22)	α_{2B}AR Cases (n=39)	α_{2B}AR Controls (n=22)	FACTOR V Cases ((n=39	FACTOR V Controls (n=22)	PROTHROMBIN Cases (n=39)	PROTHROMBIN Controls (n=22)	MTHFR Cases ((n=39	MTHFR Controls ((n=22
Normal	04 (10.2)	18 (81.9)	31 (79.5)	16 (72.7)	39 (100)	21 (95)	39 (100)	22 (100)	29 (74.4)	17 (77.3)
Heterozygous	03 (7.7)	02 (9)	04 (10.2)	05 (22.7)	00 (0)	01 (5)	00 (0)	00 (0)	09 (23)	05 (22.7)
Variant	32 (82)	02 (9)	04 (10.2)	01 (4.5)	00 (0)	00 (0)	00 (0)	00 (0)	01 (2.56)	00 (0)
Wild Type	11 (14.1)	38 (86.3)	66 (84.6)	37 (84.1)	78 (100)	43 (97.8)	78 (100)	44 (100)	67 (85.9)	39 (88.6)
Mutant Type	67 (85.9)	06 (13.7)	12 (15.4)	07 (15.9)	00 (0)	01 (2.2)	00 (0)	00 (0)	11 (14.1)	05 (11.4)

in α_{2B}AR I/D polymorphism in Saudi population OR-0.6882, p=0.9388 (95% CI=0.2035-2.327) DD genotype.

PCR-RFLP for G1691A

In the G6PD deficiency group, the GG (100%) and none of the genotype (GA and AA) were present. In the control group GG genotype was found to be 95% and GA was found to be 5%. No statistical analysis have shown any association with either allele or genotype frequencies OR=0.566, p=.0.667, (95% CI=0.014-22.48) and OR=0.569, p=0.251¸ (95% CI=0.014-22.96).

PCR-RFLP for G20210A

No significant evidence of association was observed for any of the phenotypes investigated with G6PD cases. For G Vs A, p=0.774; OR=0.566 (95%CI; 0.011-29.6); AA+GA Vs GG; p=0.502; OR=0.569 (95%CI=0.010-2969). G and A allele frequencies were similar between cases and controls with no statistical significance.

PCR-RFLP for C677T

We have compared the C677T cases with the controls. None of the genotypes or allele frequency cannot show any association OR=1.281, p=.0.667, (95% CI=0.414-3.958) and OR=1.1.172, p=0.800¸ (95% CI=0.343-4.008). Similarly, the difference of T allele frequencies between patients and controls was not found any association.

Discussion

This study is a comprehensive investigation conducted in 2011 to determine the frequency of G6PD deficiency and its variants in Saudi men. For the G6PD deficiency there are many hematological and genetic studies were conducted in the kingdom of Saudi Arabia [12]. The G6PD deficiency is one of the commonly occurring genetic enzyme disorders known to affect millions of individuals worldwide and an increased risk for venous thrombosis. It is linked to the Mediterranean basin. In G6PD deficiency males may be either G6PD normal or deficient hemizygotes, whereas females may be normal homozygotes, deficient homozygotes, or heterozygotes. In the G6PD gene 13 exons were present and complete genetic se-

quencing analysis were conducted in the Saudi Arabia individuals. Till now there are no studies were conducted on other gene polymorphisms apart from G6PD gene. In our study we have conducted I/D polymorphisms like ACE and α_{2B}AR as well as thrombophilic gene mutational studies to rule out whether G1691A, G20210A and C677T mutations may play any role in G6PD individuals.

The importance of ACE gene is a zinc metalloprotease widely distributed on the surface of epithelial and endothelial cells. ACE gene consists of 26 exons and spans 21 kb on human chromosome 17.The sequence codes a 1306 amino-acid protein including a signal peptide. The gene product of ACE is composed of two homologous domains with two active sites. It has a common polymorphism which consists of the presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence within intron 16. ACE activity in individuals with DD genotype was found to be more than 8 times in those with II genotype. Subjects with ID genotype exhibit intermediate levels of ACE [10].

α_{2B}AR I/D polymorphism is leading to a detection of three glutamic acids from a glutamic acid repeat element (Glu X 12, amino acids 297-309), located at the third intracellular loop of the receptor protein has been identified. The important selection of this polymorphism was found to be more associated with reduced basal metabolic rate in obese individuals. In this study most of the men over weight and obese. From this gene study we didn't find any association either with allele or with genotypes. The reason that the α٢BAR I/D polymorphism could not show any significant effect in G6PD individuals could be due to a much strong impact of G6PD deficiency as per compared with that of this gene variant.

FVL is a point mutation which impairs the proteolytic degradation of the factor V by protein C that makes it active for a long time. This abnormality of the hemostasis system is associated with an increased risk of venous thrombosis. The prevalence of the mutation varies among different populations, being high in Europeans and almost absent in the populations of Southeast Asia, Polynesia and Africa. The largest population study among Europeans revealed a mean frequency of 2.78% with a peak value of 12% in Cyprus with decreasing frequency from south to north and from west to east. 11% of the prevalence in G6PD deficiency was found in the Dalmatian population In an Iran the prevalence rate was 2.5% and in our population this mutation was completely absent in the G6PD deficiency [6].

Prothrombin, the precursor of thrombin is a vitamin K-dependent glycoprotein that plays a central role in blood coagulation. Activated by factor Xa, thrombin converts fibrinogen to fibrin.

The prothrombin (PRT) gene is located on chromosome 11 near the centromere, spans approximately 21 kb, and is composed of 14 exons and 13 introns PRT G20210A represents a gain-of-function mutation which affects the 3'-terminal nucleotide of the 3'– untranslated region (UTR) of the mRNA. PRT is the second most common cause of familial thrombophilia is a mutation in the 3' untranslated region of the prothrombin gene (G20210A). This mutation leads to an elevated level of prothrombin because of increased synthesis but does not lead to an altered aminoacid sequence or altered function [7]. Elevated levels of prothrombin occur due to increased cleavage site recognition, increased 3' end processing and increased mRNA accumulation and protein synthesis. Resistance to activated protein C secondary to hyperprothrombinemia has also been reported. The prevalence of this gene variant varies widely worldwide, being virtually absent in Africa, Asia, and natives of America, while being common in Europe. In the general European population the prevalence has been reported to range from 0.7% to 4%, and 5.5% in the UK. In contrast, factor II G20210A was found in only one of 441 African Americans and was absent among Japanese subjects. In unselected patients with venous thrombosis, the prevalence ranges from 4% to 8%. Abu Amero et al [2] studies have shown that 1.7% prevalence rate for G20210A mutation in the Saudi Arabia. Their sample size of overall study was 1206 which is almost all 20 times more than our samples. In our study heterozygotes and mutants were completely absent and it indicates that the person who had can be prone for the disease. G20210A mutation had no role in the G6PD deficiency [2].

MTHFR is a plasminogen activator inhibitor type 1 (PAI-1) gene mutation and factor XIII (val34leu) contribute to the thrombotic manifestations of inflammatory bowel disease. MTHFR is involved in the one-carbon cycle, which is of im-

portance for nucleotide synthesis and methylation of DNA, membranes, proteins and lipids. Most common MTHFR polymorphisms are C677T and A1298C. Mutations in this gene result in a decrease of the enzyme activity that leads to mild hyperhomocysteinemia. A study from Szczeklik A et al suggested mutations in this gene for allele C677T and A1298C to be a tendency to associate with coronary artery disease, in both homozygous and heterozygous carriers even when blood homocysteine levels were not elevated [8, 11]. The frequency of MTHFR gene mutations varies between different population and also among racial and ethnic groups. In our study group the frequency was found to be 2.5%. In the Europeans the frequency of mutant T allele is 18.6%, Asian Population is 20.8%, 32.2% in an American population, 6.6% in an African population, 4.7% in an Australasian population and 29.1% in the Iran population (3 genes Paper). The association between this variant and venous thromboembolism/coronary artery disease has been controversial. The frequency of T allele in our healthy population (0 %) when observed in European (18.6%) or the Asian population (20.8%) and lower than in the American population (32.2%). Higher prevalence of mutant T allele (29.1%) existed in G6PD deficient individuals in an Iran compared to healthy population, but was not statistically significant when compared with our study.

There are multiple studies were carried out in G6PD individuals and have been reported. The African A mutation studies and the two mutations i.e. C563T and 1311 C>T G6PD Mediterranean and G6PD Sibiri with different polymorphic rates reinstate earlier studies. The G6PD Mediterranean, having C→T transition at nucleotide 563 of exon 6 with most prevalent allele has been reported from Mediterranean Middle East and India as well [7]. This group has been carried out the genetic work on the complete exon sequence of 6 and 7 in the G6PD gene in Jeddah. G6PD gene polymorphism study was carried out in G6PD deficient in more than 20 Arab countries and none of them has been worked on I/D polymorphisms. Till date there are no I/D polymorphism studies in G6PD patients and this will be the first genetic study in G6PD individuals from the Saudi Arabia.

Frequency of G6PD deficiency in KSA is very high when compared to other study population, and still not be screening routinely for blood donor. The evolutionary conservation of a housekeeping gene such as G6PD is greater than that of tissue-specific genes, presumably because the latter may require more specific adaptation to the physiology of individual organisms. The abundance of distinct mutation sites and their clinical manifestations make G6PD ideal for structure-function analysis. In our previous study [3], we studied gene-gene and gene-root distances highlighted relatively well the feature of evolutionary data for homologous between species. The application of sequence similarity study approach and phylogenic studies as shown by this work may significantly advance the understanding of protein sequence-structure function relationships and guide experimental characterization of protein function. This analysis reveals the presence of G6PD mutated sequences among species and gene clusters provide significant differences which helps to identify the evolutionary path that might have included.

In our study we have found that ACE I/D polymorphism study was found to be strongly associated and 82% of individuals were found to be deletion of Alu sequence, which means prone for the disease and in the α۲BAR gene I/D polymorphism didn't show any association with the disease and none of the prothrombotic gene mutations are important risk factors for G6PD deficiency in the Saudi population. It indicates that these mutations cannot be used to identify patients with hematological disorders and our results are not consistent with previous findings in the Mozafari M et al studies from the western region of the Iran in an Arab population. We have mentioned one of the reasons earlier and the other reason could be because of lack of sample size. In our study we have chosen only 39 samples because all the men participated in this study is purely Saudis from Taif city. We recommend having a large sample size can help to rule out the disease whether it is showing any important role or not.

From our study we conclude that G6PD deficiency is showing strong association in DD genotype and D allele of ACE gene. Any individual effecting DD genotype and D allele is prone for the disease in ACE gene. We could not able to show any association with other genes selected in this study. Overall, our analysis reveals the pre-

sence of G6PD mutated sequences among species and gene clusters provide significant differences which helps to identify the evolutionary path that might have included. This analysis highlights the conserved presence as well as functional importance of G6PD revealed by mutational studies. In the present case, gene-gene and gene-root distances highlighted relatively well the feature of evolutionary data for homologous between species.

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Foodborne type B botulism in two nurses from Western Romania

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Abstract

Background: Botulism is a fatal illness if not diagnosed early and treated adequately.

Aim: The aim of present study was to evaluate the clinical and evolutive patterns of foodborne botulism identified.

Material and Methods: We retrospectively analysed data from the medical files of 2 botulism cases. A positive diagnosis was confirmed by epidemiological factors, clinical presentations and specific bioassay results for identification of botulinum neurotoxin.

Results: Serum samples tested positive for type B botulinum toxin. Under treatment with polyvalent antibotulinum serum evolution was positive for both cases.

Conclusion: Consumption of improperly canned and stored food may lead to botulism/food poisoning.

Key words: foodborne botulism, anti-botulinum serum, nurses, food safety

Introduction

Botulism is a neuroparalytic illness caused by neurotoxins of *Clostridium botulinum* and rare strains of *C. butyricum* and *C. baratii.* Dormant *C. botulinum* spores germinate and produce toxins (types A–G) under the rare confluence of anaerobic conditions, low acidity (pH >4.5), low salt and sugar concentrations and a temperature >10°C (for most strains). These toxins irreversibly bind to presynaptic nerve endings and inhibit acetylcholine release, resulting in cranial neuropathy and symmetric descending flaccid paralysis, which may progress to respiratory failure (1).

In the United States, the median number of foodborne botulism cases per year between 1990 and 2000 was 23 (range, 17–43 cases). Most cases were sporadic and outbreaks typically involved only 2–3 people; however, larger outbreaks caused by commercially prepared foods have also been reported (2).

The median annual incidence rate of botulism in Romania was 0.05 per 100 000 persons during 1980–1989, which increased to 0.06 per 100 000 during 1990–1998 and to 0.09 per 100 000 during 1999–2006 (3). Thus, botulism is becoming a life-threatening disease. Here we report the clinical and epidemiological peculiarities of foodborne botulism diagnosed in 2 nurses working in a town hospital in Romania.

Materials and methods

We retrospectively analysed data from the medical files of 2 type B botulism cases diagnosed at the Infectious Disease Clinic in Timisoara, Romania, in 2011. A positive diagnosis was confirmed by epidemiological factors (our 2 cases of toxic food infection occurred after consumption of liver pastry from the same can), clinical presentations (nausea, dysphagia, belching, cephalalgia, asthenia as well as nasal, oral and pharyngeal mucous dryness, foggy vision, dysphonia, deglutition disorders, near sight impairment, bloating, constipation, etc.) and specific bioassay results for detection and identification of botulinum neurotoxin.

To identify the botulinum neurotoxin, serum samples were obtained from both patients on the first day of hospitalization, immediately before initiation of therapy with polyvalent anti-botulinic serum. Both cases were reported to the Public Health Department of Timis County, Romania, as foodborne botulism within the first 48 h of patient hospitalization. The local public health department ordered an epidemiological investigation into these cases of botulism.

Clinical diagnosis was confirmed by the intraperitoneal inoculation test performed using mice for detection of botulinum toxin in the serum, and the seroneutralization test was performed with types A, B and E anti-botulinum sera (bioMérieux, Marcy l'Etoile, France) to determine the exact type of botulinum toxin. The tests were performed at the National Institute of Research and Development for Microbiology and Immunology „I. Cantacuzino" (Bucharest, Romania). Data extracted from the medical files of the patients were analysed using the Microsoft Excel data analysis program (Microsoft Corp., Redmond, WA, USA).

Results

The first patient hospitalized was a 35-year-old female who lived in an urban area, while the second was a 22-year-old female from a rural area. Both women worked as registered nurses at the Military Hospital in Timisoara, Romania. On July 26, 2011, they both ate liver pastry from the same can stored at room temperature (25°C). The incubation period was 3 and 4 days, respectively. The following symptoms appeared while the patients were still in good health: mucous membrane dryness, nausea, dysphagia, cephalalgia, belching, asthenia, bloating and constipation followed by phonation, deglutition and diplopia with distance ocular accommodation disorders (Table 1).

Neurological examinations confirmed phonation and deglutition disorders, diplopia and distance ocular accommodation dysfunction. Symptoms such as impaired throat reflex, decreased photomotor reflex, dysphonia, deglutition disorders and mydriasis appeared within 4 days of consumption of the liver pastry.

Serum analysis showed normal concentrations of white blood cells (6330 ± 1315.21/uL), haemoglobin (13.5 ± 12.02 g/dL), alanine transaminase (21.5 ± 12.02 U/L), aspartate aminotransferase (24.5 ± 0.70 U/L), sanguine urea (24.5 ± 2.12 mg/dL) and creatinine (0.5 ± 0.70 mg/dL). Analysis of pharyngeal exudates was negative in both patients.

The intraperitoneal inoculation test was performed using mice to detect botulinum toxin, which was positively identified in the samples from both patients. Furthermore, the seroneutralization test performed with types A, B and E anti-botulinum sera revealed the presence of botulinum toxin type B in both cases.

The first patient was examined in our ophthalmology clinic and then sent to the neurology clinic for further evaluation. The neurologists sent the patient to an infectious disease clinic where she was hospitalized after 4 days. The following day, the second patient required hospitalization at the recommendation of the infectious diseases specialist. Both patients responded well to treatment with polyvalent anti-botulinum serum (A, B and C), hydrocortisone hemisuccinate, omeprazole, metoclopramide and B vitamins.

To identify potential allergic reactions to treatment, 1 vial (10 mL) of trivalent equine anti-toxin

Table 1. Reported symptoms and physical signs

Symptoms	No. of cases	Clinical signs	No. of cases
Nausea	1	Dry teguments	2
Dysphagia Diplopia	2 2	Dehydrated mucous membranes	2
Belching	1	Conscious patient	2
Cephalalgia	1	Space and time oriented	2
Asthenia	2	Decreased throat bottom reflex	2
Nasal, oral and pharyngeal mucous membrane dryness	2	Decreased photomotor reflex	2
Foggy vision	2	Moderate mydriasis	2
Near sight impairment	2		2
Dysphonia	2		
Deglutition disorders	2		
Bloating	1		
Constipation	2		

(ABE) containing 5500–8500 IU of antibodies of each type was diluted (1:10) with 0.9% saline solution, and 0.1 mL of the dilution was intradermally administered to the patients. After 20 min, a second 0.1-mL dose was administered subcutaneously. Because neither woman showed an allergic response, the remaining trivalent anti-botulinic serum was administered intramuscularly. The patients were clinically observed during therapy and arterial blood pressure, heart rate, body temperature, electrocardiogram readings and blood oxygen saturation were recorded in the patient charts. Of note, no local or systemic secondary reactions occurred in either patient after administration of the trivalent anti-botulinic serum. Furthermore, both patients were apyretic and conscious and displayed no severe infectious respiratory or psychiatric complications.

Discussions

Botulism is a rare disease with 4 naturally occurring syndromes: foodborne botulism caused by ingestion of foods contaminated with botulinum toxin, wound botulism caused by *C. botulinum* colonization of a wound and in situ toxin production, infant botulism caused by intestinal colonization and toxin production and adult intestinal toxemia botulism, which is a rare form of intestinal colonization and toxin production (10). All forms of botulism produce the same distinct clinical syndrome of symmetrical cranial nerve palsies followed by descending, symmetric flaccid paralysis of voluntary muscles, which may progress to respiratory distress and death (1).

Foodborne botulism is a severe paralytic illness caused by consumption of food that contains botulinum toxin, a product of *C. botulinum*. The acute illness is well-described and involves cranial nerve dysfunction, symmetric descending flaccid paralysis and potential respiratory failure that can last for months until nerve endings regenerate (4).

The standard test for laboratory diagnosis and confirmation is a bioassay involving intraperitoneal injection of toxin into mice and observation of the development of botulism-specific symptoms. Toxin type is determined by injecting a panel of mice with mixtures of the test sample and a specific monoclonal anti-toxin (e.g. anti-A or anti-B) and by observing which anti-toxin confers protection to the mice (1).

Because botulism is a life-threatening condition, rapid diagnosis is essential and relies on clinical observation and laboratory analysis to subsequently confirm or refute the clinical diagnosis (5). Both of our patients were treated with polyvalent anti-botulinum serum before receiving the test results for the determination of the type of botulinum toxin. The advanced administration of anti-botulinum serum was very likely to have influenced the positive evolution of the 2 cases with mild forms of cranial nerve paralyses. The only specific treatment for botulism is the administration of botulinum anti-toxin, which can arrest the progression and limit the duration of paralysis and decrease the dependence on mechanical ventilation. Anti-toxin should be administered early in the course of illness, ideally <24 h after the onset of symptoms (6,7), because anti-toxin neutralizes only toxin molecules that are yet unbound to nerve endings (8).

Extra-ocular muscle paralysis is due to the paralysis of cranial nerves III, IV and VI and manifests as blurry vision or diplopia and impairs near vision. Ptosis is prominent (8). Paralysis of cranial nerve VII produces expressionless facies, and dysphagia is caused by cranial nerve IX paralysis, which may present as regurgitation of masticated food or beverages; dysarthria is prominent. Prominent autonomic symptoms include anhidrosis with severe dryness of the mouth and throat and postural hypotension (8).

C. botulinum is ubiquitously found in soil and aquatic sediments and produces 7 immunologically distinct toxins (A–G) that cause clinically similar, highly recognizable syndromes. Human cases are mostly caused by toxin types A, B, E and (rarely) F (1).

C. botulinum infections occur worldwide, but botulism is difficult to diagnose. The botulinum toxin is not always detectable in food samples. The type A botulinum toxin occurs more frequently in soils in the United States, whereas type B is more frequently found in Europe. Baltic countries display the highest level of type E contamination in the world (9). From 2006 to 2008, there were 477 confirmed cases in the European Union, which equates toan average of 119 (range, 104–132) cases per year, but no discernible trend has been identified (11).

From 2003 to 2008, in Romania, botulinum toxin was detected in 80 serum samples (79 type

B and 1 type E): 27 cases (13%) in 2003, 18 (9%) in 2004, 21 (10%) in 2005, 23 (11%) in 2006, 110 (52%) in 2007 and 11 (5%) in 2008 (9). The 2 cases of type B foodborne botulism presented in this article confirm the predominance of type B botulism in Romania. Under stress, *C. botulinum* forms a spore that survives standard cooking and food-processing measures. However, the confluence of conditions permitting spore germination (i.e. anaerobic milieu, nonacidic pH and low salt and sugar content) is rarely achieved in food, which explains the small number of foodborne botulism cases (1).

Most food borne botulism outbreaks in Romania are attributable to improperly home-canned foods, including liver, fish, vegetables, chicken products, pork and other marine species (9). The type B botulinum toxin is most often isolated from food, although type E is most often isolated from fish products (9).

The present cases of foodborne botulism occurred in 2 nurses who consumed liver pastry from the same can without checking the expiration date. The can was purchased from a supermarket 2 months prior to infection but was not kept refrigerated; rather, it was kept in a kitchen cupboard for the entire summer. Unfortunately, the remaining liver pastry was not preserved, and we were unable to perform testing to demonstrate the etiological agent.

The epidemiology specialists from the local Public Health Department notified the Consumer Protection Association to recall cans of the implicated liver pastry in Timisoara to prevent the occurrence of further food poisoning.

It is necessary to educate the population regarding compliance with food storage standards, especially during the summer months, and to reiterate the importance of reading the expiration date printed on food containers before consuming the contents. This is the first report regarding type B foodborne botulism in medical personnel after canned liver pastry consumption.

In adults, botulism results in pulmonary complications in 81% of patients, with respiratory failure in one-third. Mortality in foodborne botulism reaches 5-10% treated and 60% untreated patients (12). The 2 present cases displayed clinical symptoms and objective changes specific to foodborne botulism and were confirmed through specific biological tests. The early administration of polyvalent anti-botulinic serum contributed to the favourable treatment outcomes in both patients, who experienced no complications such as airway obstruction or respiratory muscle paralysis that would require endotracheal intubation or mechanical ventilation.

Cases of foodborne botulism are a medical emergency requiring the administration of pathogenic therapy before confirmation of the diagnosis by specific serological tests (10). Rapid diagnosis, provision of intensive care and administration of botulinum anti-toxin are the cornerstones of treatment. Anti-toxin is available exclusively from public health authorities, who immediately investigate potential sources to prevent additional illnesses (10).

Conclusions

Consumption of improperly canned and stored food may lead to botulism/food poisoning. Early therapy with polyvalent serum anti-botulinic can contribute to the development of favourable treatment outcomes and prognoses of patients with foodborne botulism.

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Clinical severity of sickle cell diseases in genders

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Abstract

Background: We tried to understand whether or not there is a gender difference in severity of sickle cell diseases (SCDs).

Methods: All SCDs patients with red blood cell (RBC) transfusions of less than 10 units in their lives were put into the first, 10 units or higher were put into the second, and 50 units or higher were put into the third groups.

Results: The study included 284 patients. Mean ages of the groups were similar (28.8, 28.8, and 29.6 years, respectively). Prevalences of cases without any RBC transfusion in their lives were 6.4% and 2.7% in females and males, respectively ($p<0.05$). Prevalences of cases without any painful crisis were 14.3% and 6.2% in females and males, respectively ($p<0.001$). There were progressive increases according to mean painful crises per year, smoking, digital clubbing, chronic obstructive pulmonary disease (COPD), leg ulcers, stroke, chronic renal disease (CRD), pulmonary hypertension, and male ratio from the first towards the third groups ($p<0.05$, nearly for all). Mean ages of the mortal cases were 31.0 and 25.7 years in females and males, respectively ($p>0.05$).

Conclusion: Although strong atherosclerotic effects of smoking, significantly higher painful crises per year, digital clubbing, COPD, leg ulcers, stroke, CRD, pulmonary hypertension, and male ratio of the third group, lower mean ages of male SCDs patients with mortality, lower male ratio of patients without any RBC transfusion, lower male ratio of patients without any painful crisis, and longer overall survival of females in the world could not be explained by smoking alone.

Key words: Sickle cell diseases, smoking, atherosclerosis, metabolic syndrome.

Introduction

Atherosclerosis may be the major pathology of the human being, and the systemic process, probably affecting whole arterial vasculature, is the major cause of aging (1,2). Atherosclerosis is an irreversible process initiating at birth, and is accelerated by many factors. Probably just a minority of the accelerating factors are known for today, and they are collected under the heading of metabolic syndrome. Some of the components of the syndrome known for today are overweight, dyslipidemia, white coat hypertension, insulin resistance, and smoking for the development of eventual consequences including obesity, diabetes mellitus (DM), hypertension (HT), coronary heart disease (CHD), chronic obstructive pulmonary disease (COPD), cirrhosis, chronic renal disease (CRD), peripheric artery disease, and stroke (3-9). Sickle cell diseases (SCDs) are a prototype of the accelerated systemic atherosclerotic process (10-13), and they are characterized by sickle-shaped erythrocytes which is caused by homozygous inheritance of the hemoglobin S (Hb S). As a less polar amino acid in the sixth position of beta chain of the Hb S, glutamic acid is replaced with valine. Presence of the less polar amino acid promotes polymerisation of the Hb S. So Hb S causes erythrocytes to change their normal elastic structures to hard bodies. The rigidity of erythrocytes is the central pathology of the diseases. The sickling process is probably present in whole life, but it is exaggerated with various stresses. The erythrocytes can take their normal elastic shapes after normalization of the stressful conditions, but after repeated cycles of sickling and unsickling, they become hard bodies, permanently. The sickled cells induced chronic endothelial damage causes tissue ischemia, infarctions, and end-organ failures, even in the absence of obvious vascular occlusions in the chronic background of the SCDs (14).

We tried to understand whether or not there is a gender difference in clinical severity of SCDs in the present study.

Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and March 2013. All patients with SCDs were enrolled into the study. SCDs are diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography (HPLC). Their medical histories including numbers of painful crises per year, units of transfused RBC in their lives, regular alcohol consumption, smoking habit, leg ulcers, and stroke were learnt. Cases with a history of three pack-year were accepted as smokers. A check up procedure including serum iron, total iron binding capacity, serum ferritin, serum creatinine value on three occasions, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, an electrocardiography, an abdominal ultrasonography, a Doppler ultrasonography to evaluate the portal blood flow, an endoscopy to detect esophageal varices just in suspected cases, and a computed tomography of the brain was performed. Cases with acute painful crisis or any other inflammatory event were treated at first, and then the spirometric pulmonary function tests to diagnose COPD, the Doppler echocardiography to measure the systolic blood pressure (BP) of pulmonary artery, renal and hepatic function tests, and measurement of serum ferritin level were performed on the silent phase. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% (15). Systolic BP of the pulmonary artery of 40 mmHg or higher during the silent phase is accepted as pulmonary hypertension (16). CRD is diagnosed with a permanently elevated serum creatinine level of 1.3 mg/dL or higher on the silent phase. Cases with renal transplantation were put into the CRD group. Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, esophageal varices, and ascites without any histologic procedure in the absence of indication. Digital clubbing is diagnosed by determining of the ratio of distal phalangeal diameter to interphalangeal diameter which is required to be higher than 1.0, and with the presence of Swamroth sign (17,18). Associated thalassemias are detected by serum iron, total iron binding capacity, serum ferritin, and the hemoglobin electrophoresis performed via HPLC. A stress electrocardiography was performed in cases with an abnormal electrocardiography and/or history of angina pectoris. A coronary angiography was obtained just for the stress electrocardiography positive cases. So CHD was diagnosed, angiographically. Eventually, cases with RBC transfusions of less than 10 units in their lives were put into the first, 10 units or higher were put into the second, and 50 units or higher were put into the third groups, and the three groups were compared in between. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 284 patients with SCDs (145 males and 139 females). There were 90 cases (31.6%) in the first, 105 cases (36.9%) in the second, and 89 cases (31.3%) in the third groups without any significant difference in distribution ($p>0.05$). There was not a significant difference according to the prevalence of associated thalassemias between the three groups, either (Table 1). Mean ages of the three groups were similar, too (28.8, 28.8, and 29.6 years, respectively, $p>0.05$ between all). The mean units of transfused RBCs were 4.1, 21.3, and 95.9, respectively ($p<0.001$ between all). There were 13 cases without any RBC transfusion in their lives with a mean age of 27.9 ± 6.6 (17-39) years, and the prevalences were 6.4% and 2.7% in females and males, respectively ($p<0.05$). The mean age of cases with RBC transfusion or transfusions in their lives was 29.3 ± 9.3 (13-59), and the difference between the cases with and without RBC transfusion or transfusions was nonsignificant ($p>0.05$). There were 29 cases without any painful crisis in their lives with a mean age of 35.9 ± 9.7 (18-58) years, and the prevalences were 14.3% and 6.2% in females and males, respectively ($p<0.001$). Interestingly, the mean age of cases with painful crises in their lives was 28.5 ± 8.9 (13-59), and it was significantly lower than the cases without any crisis in their lives ($p<0.000$). There was a progressive and si-

gnificant increase according to the male ratio from the first towards the third groups (37.7%, 49.5%, 66.2%, $p<0.05$ between all). There was not any patient with regular alcohol consumption among the study cases. Although the prevalences of cirrhosis, CHD, and exitus were similar in the three groups ($p>0.05$ between all), there were progressive and significant increases according to mean painful crises per year, smoking habit, digital clubbing, COPD, leg ulcers, stroke, CRD, and pulmonary hypertension from the first towards the third groups ($p<0.05$, nearly in all steps) (Table 2). Mean ages of the mortal cases were 31.0 ± 10.6 (19-45) and 25.7 ± 6.3 (19-39) years in females and males, respectively ($p>0.05$). On the other hand, five of the CRD cases were on hemodialysis, and one with renal transplantation. Although antiHCV was positive in two of the cirrhotics, HCV RNA was detected as negative by polymerase chain reaction in both. Histological diagnosis of cirrhosis was required in none of the study cases.

Table 1. Sickle cell patients with the units of red blood cell transfusions

Variables	Cases with RBC* transfusions of less than 10 units	p-value	Cases with RBC transfusions of 10 units or higher	p-value	Cases with RBC transfusions of 50 units or higher	p-value†
Prevalence	31.6% (90)	ns‡	36.9% (105)	ns	31.3% (89)	ns
Thalassemia minors	45.5% (41)	ns	42.8% (45)	ns	41.5% (37)	ns
Mean RBC units	4.1 ± 2.5 (0-9)	<0.001	21.3 ± 10.0 (10-47)	<0.001	95.9 ± 51.5 (50-264)	<0.001
Mean age (year)	28.8 ± 10.1 (13-59)	ns	28.8 ± 8.6 (15-56)	ns	29.6 ± 8.8 (14-56)	ns
Male ratio	37.7% (34)	<0.05	49.5% (52)	<0.01	66.2% (59)	<0.001

**Red blood cell*
†Difference between the first and third groups
‡Nonsignificant (p>0.05)

Table 2. Sickle cell patients with associated disorders

Variables	Cases with RBC* transfusions of less than 10 units	p-value	Cases with RBC transfusions of 10 units or higher	p-value	Cases with RBC transfusions of 50 units or higher	p-value†
Painful crises per year	2.0 ± 3.8 (0-24)	0.000	4.2 ± 6.1 (0-36)	0.011	7.0 ± 8.8 (0-36)	0.000
Smoking	4.4% (4)	ns‡	3.8% (4)	<0.001	13.4% (12)	<0.001
Digital clubbing	4.4% (4)	ns	3.8% (4)	<0.001	11.2% (10)	<0.01
COPD§	1.1% (1)	ns	0.9% (1)	<0.001	14.6% (13)	<0.001
Leg ulcers	7.7% (7)	ns	11.4% (12)	<0.01	22.4% (20)	<0.001
Stroke	4.4% (4)	ns	3.8% (4)	<0.001	12.3% (11)	<0.001
CRD¶	1.1% (1)	<0.001	8.5% (9)	<0.05	14.6% (13)	<0.001
Pulmonary hypertension	5.5% (5)	<0.001	16.1% (17)	ns	11.2% (10)	<0.05
Cirrhosis	3.3% (3)	ns	4.7% (5)	ns	2.2% (2)	ns
CHD¶	8.8% (8)	ns	8.5% (9)	ns	8.9% (8)	ns
Exitus	3.3% (3)	ns	6.6% (7)	ns	3.3% (3)	ns

**Red blood cell*
†Difference between the first and third groups
‡Nonsignificant (p>0.05)
§Chronic obstructive pulmonary disease
¶Chronic renal disease
***Coronary heart disease*

Discussion

Acute painful crises are nearly pathognomonic symptoms of the SCDs. For example, only 10.2% of the study cases have not had any crisis in their lives in the present study. The mean age of cases with crises was significantly lower than the cases without any painful crisis in their lives (28.5 versus 35.9 years, $p<0.000$). So painful crises may indicate clinical severity of the SCDs, and the prevalences of cases without any crisis were significantly lower in males (14.3% versus 6.2%, $p<0.001$). Although the crises may not be life threatening, directly (19), infections are the most common precipitating causes of them. SCDs cases are immunocompromised due to a variety of reasons including a functional and anatomic asplenism, chronic endothelial damage induced end-organ insufficiencies, a permanent inflammatory process all over the body, and frequent hospitalizations, transfusions, and invasive procedures. Because of the repeated infarctions and subsequent fibrosis during early years of life, a functional and anatomic asplenism develops with the decreased antibody production, prevented opsonization, and reticuloendothelial dysfunction in SCDs patients. Terminal consequence of the asplenism is an increased risk of infections, particularly with Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis like encapsulated bacteria. Thus, infections especially pneumococcal infections are common in early childhood, and are associated with a high mortality rate. The causes of death were infection in 56% of infants in a previous study (20). In another study, the peak incidence of death occured between 1 and 3 years of age in children, and the deaths were predominantly caused by pneumococcal sepsis in patients younger than 20 years of age (21). As also observed by us in the present study that SCDs cases, even those who appear relatively fit, are susceptible to sepsis induced multiorgan failure and sudden death during acute painful crises due to the relative immunosuppression.

Probably pain is the result of disseminated tissue hypoxia secondary to the chronic endothelial inflammation on the background of interactions between sickled cells, endothelial cells, leukocytes, and platelets. The adverse actions of platelets and neutrophils on endothelium are of particular interest in SCDs cases. For instance, leukocytosis even during silent periods was an independent predictor of the severity of the SCDs (20), and it was associated with increased risk of stroke (22). On the other hand, leukocytosis and thrombocytosis are acute phase reactants that are nearly present in all SCDs patients even during the silent periods. They indicate presence of a permanent inflammatory process initiating at birth. The continuous inflammatory process alone causes an additional accelerated atherosclerotic process, and a relative weight loss in the SCDs cases (23). So the mean weight and body mass index (BMI) were significantly retarded in the SCDs cases (19). On the other hand, as an opposite finding to some reports (24-25), the mean heights were similar in the SCDs with the controls in the above study (23). Probably due to the significantly lower mean weight and BMI, mean values of the low density lipoprotein cholesterol, alanine aminotransferase, and systolic and diastolic BPs were also lower in the SCDs cases (23), which can be explained by definition of the metabolic syndrome (26-27).

Multiorgan failures are not unusual, and the risk of mortality is significantly higher during the painful crises of SCDs patients. During severe crises, RBC transfusions may provide adequate tissue oxygenation and immunity, and prevent intractable pain, dissemination of infections, and end-organ failures. Due to the severity of pain, narcotic analgesics are usually required to control them (28), but according to our practice, simple RBC transfusions may be highly significant during severe crises, both to relieve pain and prevent sudden deaths secondary to multiorgan failures developed on the chronic background of SCDs. Although analgesics may relieve pain alone, they can not reverse the underlying destructive process, but RBC transfusions can. So frequency of RBC transfusions may be another indicator of clinical severity, and the prevalences of cases without any RBC transfusion in their lives were also lower in males, significantly (6.4% and 2.7%, $p<0.05$).

The sickled cells induced chronic endothelial damage causes tissue ischemia, infarction, and end-organ failures, so SCDs affect all organ systems where the vascular endothelium is present in the body (29-30). Even there were patients with

severe vision or hearing losses among our study cases. For example, digital clubbing and recurrent leg ulcers may indicate chronic tissue ischemia. The chronic endothelial inflammation, initiating at birth, may cause swelling of the endothelium, and prevents passage of blood to the tissues, sufficiently, even in the absence of obvious vascular occlusions. Eventually, the mean survivals were 42 and 48 years for males and females in the literature (14), whereas the mean ages of mortal cases were 25.7 and 31.0 years in males and females in the present study, respectively. The great differences between the survival may be secondary to the initiation of hydroxyurea treatment in infancy in the developed countries (12). Although the lower mean age of mortal cases with SCDs in males, the difference was nonsignificant probably due to the small number of cases in the present study (p>0.05). As a similar result to above ones, females have a longer overall survival than males in the world (31).

As a conclusion, although the already known strong atherosclerotic effects of smoking, the significantly higher painful crises per year, digital clubbing, COPD, leg ulcers, stroke, CRD, pulmonary hypertension, and male ratio of the third group, the lower mean ages of the male SCDs patients with mortality, the lower male ratio of patients without any painful crisis in their lives, the lower male ratio of patients without any RBC transfusion in their lives, and the longer overall survival of females in the world could not be explained by the effects of smoking alone, and should be searched with further studies.

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Which method has less error in measuring root canal curvature? Digital or manual?

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Abstract

Introduction: Research about root curvature is very important since it can affect procedural accidents during root canal treatment and its prognosis. So the present study aimed to evaluate the error rate between manual and digital methods in root canal curvature measurement.

Materials and methods : The printed and LCD periapical radiographs of 260 patients who need root canal treatment for their maxillary first molar and referred to a private endodontic clinic in Babol from January 2011 to February 2012, were studied. The radiographs were taken using periapical parallel technique. A maxillofacial radiologist and an endodontist measured the degree of root canal curvature separately. In the manual method, the curvature angle of mesiobuccal root of maxillary first molar was measured by Schneider technique using a conveyor .In the digital method, the measurement was conducted by Scanora 4.3.1 software (Soredex, Finland) on LCD monitor. Then data were analyzed by $SPSS_{17}$ statistical software, paired t-test and Pearson correlation coefficient.

Results: The agreement between observers was assessed using Pearson Correlation Test. There were no significant differences between observers. ($P> 0.05$) The curvature angle of mesiobuccal root of maxillary first molar in digital method was $17/11 \pm 7/79$ and in manual method was $12/68 \pm 7/73$ and a statistically significant difference was found between them. ($P <0.001$) Error rate in the digital method was 0.08 ± 0.79 and 0.37 ± 0.16 in the manual method with no significant difference. ($P> 0.05$)

Conclusions: In the present study, there was no significant difference in the error rate between digital and manual methods.

Key words: Root canal curvature, digital measurement, manual measurement.

Introduction

Morphologic and anatomic evaluation of the teeth has been a subject of great attention by many researchers over the last few years.[1,2,3,4] Study on root curvature is very precise because it can affect procedural accidents during root canal treatment, determination of working length, instrumentation difficulties, and penetration depth. [5]

There are various methods for this evaluation, among which tooth preparation, dye injection and clearing, taking radiographs of the extracted teeth, and taking radiographs during treatment can be enumerated.[4,6] In the measurement of root canal curvature, Schneider[7], Wein[8,9], and longitudinal axis[10] techniques are worth to be pointed out, all of which are somehow associated with radiography.

With new progressing and use of digital system in the measurement of root canal curvature, there is always one question about the rate of error of this measurement technique. Till now there is no study about the agreement between digital and manual methods or the rate of error between them.

So, the aim of this study was to determine the error rate between manual and digital methods in root canal curvature measurement.

Materials and methods

From a total of 486 patients who need maxillary first molar root canal treatment and referred to a private endodontic clinic in Babol from January 2011 to February 2012, 260 patients were included in the study. Inclusion criteria consisted

of the maxillary first molar teeth that required root canal treatment with complete apical foramen and contour. Teeth with extensive caries or large restorations or patients with gag reflex or having anatomic limitations for taking radiographs with paralleling techniques were excluded from the study.

Firstly, each patient was given information about the study, and a consent form was completed afterward. Then, the radiographs were taken using the periapical parallel technique with XCP film holder (Dentsply, United Kingdom) and PSP receptor (Digora Optime, Soredex, Finland) with pixel size of 40μ and spatial resolution of 12.5 LP/mm. Digital images were saved in JPEG format and size of 24.3 KB. This investigation was performed in two steps; at first, mesiobuccal canal curvature was measured on the printed film (manual method); then, after 2 weeks the measurement was carried out on the LCD monitor (digital method).In the end, for evaluating the error rate intra observers ,10 % of radiographs were measured again one month later (In both methods).

Radiographic evaluation

Each of the printed and LCD images were assigned a specific code. A maxillofacial radiologist and an endodontist measured the degree of root canal curvature separately.

In the manual method, root curvature angle was measured by Schneider technique[4, 7] on the printed images using a conveyor (Rotting, Germany) in a semi dark room on the view box.[11]

In the digital method, measurement was conducted by Scanora 4.3.1 software (Soredex, Finland) on a 19 inch LG monitor (Flatron W2053 S) with high resolution in quiet dark room.[11]

Then, the data were analyzed by $SPSS_{17}$ statistical software, paired t-test and Pearson correlation coefficient.

Results

This cross-sectional study was conducted on 260 patients (45% male and 55% female) with the mean age of 32.52 ± 11.60 years.

The inter- and intra-observer relationship was assessed by the Pearson correlation test and no significant difference was found (P> 0.05) (Table 1).

Table 1. Shows consensus between the observers

	Intra observer	Inter observer
Digital	0.999	0.996
Manual	0.999	0.999

Then the mean measurements by two observers were evaluated. The degree of mesiobuccal maxillary first molar root curvature angle was 17.11 ± 7.79 and 12.68 ± 7.73 in digital and manual methods respectively, between which a statistically significant difference was found (P <0.001)

The error rate was 0.08 ± 0.79 in digital method and 0.16 ± 0.37 in manual method, and no significant difference was observed.

Discussion

In the present study, the error rate between manual and digital methods in root canal curvature were investigated and we found no significant difference between them.

Ilguy[12] et al. stated in caries detection the quality of images on both computer and medical LCD monitors was comparable with D-speed film images.

Tofanghchiha[13] et al. also concluded there was no different significant between monitor and film (E-speed) in detection of vertical root fracture .They also suggested that the type of monitor (conventional or medical) had not influence on diagnosis of vertical root fracture but, medical monitor was more sensitive and specific than conventional monitors.

There are many studies related to comparing printed film and monitor display in dentistry.[12, 13, 14, 15] All of them emphasized on methods facilitation and advantages of the technologies. Several studies [2, 4, 5] proposed the use of periapical radiography for the detection of canal curvature. The method used in this study was based on the Schneider technique[4,7] on periapical radiograph and its measurement was carried out on the monitor screen and manually on the printed film.

Huang[16]et al. also used on-screen measurement for determining measurement accuracy in mandibular incisor. They found that curved canal length should be estimated with 3-click measurement for increasing precision, but he didn't compare the root canal curvature on-screen and on printed film images. Our results showed there was a statistically

significant difference between digital and manual methods ($p<0.001$) .In this study for approaching to clinical situation, we performed it invivo but this situation impose special problems such as absence of gold standard and sometimes patient poor cooperation for taking parallel radiographs.

In relation to error rate, there was no significant difference between the digital and the manual methods, but its amount was lower for the digital method. Maybe less involvement of clinician in reading the amount of angle causes this lowering; however, this difference was not significant.

This finding is in accordance with Schulze et al.'s study[17]. They mentioned there were no significant differences between printed images and monitor viewing for caries detection. Tofanghchiha[13] also didn't find differences between printed images and monitor viewing in vertical root fracture detection.

Until now we didn't find any article similar to our study about root canal curvature. As radiograph presents a two-dimensional image of a three-dimensional object [18],it can cause doubt in identifying the actual direction and degree of root canal curvature[19].So,we suggest to conduct this study with advanced technique such as CBCT and in vitro with more samples.

Conclusions

In the present study, there was no significant difference in error rate between the digital and the manual methods in root canal curvature measurement.

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The condition of the periodontium in patients with diabetes undergoing sanatorial treatment

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Abstract

Aim: Whether there are differences in the condition of the periodontium in patients with diabetes mellitus type 1 and 2. Whether the type of diabetes mellitus has a considerable influence on the condition of the periodontium.

Materials and Methods: The research encompassed 300 patients with diabetes mellitus aged 55,3±12,0 (from 17 to 85), including 154 female and 146 male patients with diabetes. The research results were submitted to statistical analysis on the basis of the chi-squared test and Spearman's rank correlation.

Results: Diabetes mellitus type 2 was statistically significantly higher ($p < 0,05$) in patients over 40 years of age (98.16% of the subjects). The research also showed that treatment of the periodontium of patients rarely visiting sanatoria was statistically significantly ($p<0,05$) more frequently needed.

Conclusions: No statistically significant interrelation between the condition of the periodontium, the level of compensation of diabetes mellitus and its duration was ascertained. No interrelation between the subjects' condition of the periodontium and the type of diabetes mellitus was observed. The number of stays in a sanatorium had a positive, statistically significant influence on the periodontium.

Key words: Diabetes mellitus, periodontal diseases.

Introduction

Diabetes mellitus is a disease resulting from an absolute or relative insulin deficiency. These deficiencies cause serious disorder of carbohydrate, protein and lipid metabolism, as a result of which, there are lesions in a number of essential organs, including the masticatory organ. According to Ahmed [1], the most common complications of diabetes mellitus in the oral cavity are lesions of the periodontium, whose incidence is determined at 3% in the general population. Among children with type 1 diabetes mellitus from 10-18 years of age, the incidence is of the order of 10%, whereas over 19 years of age, it is of the order of 40% [1]. According to the latest research, diabetes mellitus is not a disease that causes periodontal diseases, it is only conducive to periodontal disease progression and modifies its course. These lesions are also a result of a damage to blood vessels, which are referred to as microangiopathies. Lesions in vessels of the gingival margin hinder oxygen diffusion, impair removal of metabolic waste products and leukocyte migration, which, as a result, reduces the periodontal tissue's ability to regenerate, and increases susceptibility to damage [2-12]. Exacerbation of periodontitis in patients with diabetes mellitus may also be a result of increased activity of collagenase and, consequently, leads to destructive processes. An increased level of collagenase involves presence of gram-negative bacteria and Capnocytophaga in the dental plaque. Leukotoxins produced by these bacteria may secondarily impair activity of polymorphonuclear leukocytes [6,12,13-14].

The aim of the research was to determine:

1. Whether there are differences in the condition of the periodontium in patients with diabetes mellitus type 1 and 2.
2. Whether sanatorial treatment affects the condition of the oral cavity.
3. Whether the type of diabetes mellitus has a considerable influence on the condition of the periodontium.

Materials and methods

The research encompassed 300 patients with diabetes mellitus aged 55,3±12,0 (from 17 to 85), including 154 female and 146 male patients staying in a sanatorium for patients with diabetes. The patients were divided into two age groups: up

to 39 years of age (11% of the subjects) and from 40 years of age (89% of the subjects). The condition of the periodontium was evaluated according to Bellini's classification. The type of diabetes mellitus (1or 2), its duration and course were also assessed. Among 300 patients, type 1 was reported in 83 patients (27.7%), and type 2 in 217 patients (72.3%). Labile diabetes mellitus was reported in 170 patients (56.7%); compensated form of diabetes was observed in 130 patients (43.3%). The duration of the disease was defined in two groups: up to 15 years – 220 patients (73.3%), and over 15 years – 80 patients (26.7%). The number of patients' stays in a sanatorium was divided into three categories:

- once – 96 (32%)
- 2-5 times – 139 (46.3%)
- > 5 times – 65 (21.7%)

The frequency of treatment in a sanatorium for patients with diabetes was evaluated as well. It was divided into three groups:

- every 2 years – 101 (33.7%)
- every 2-5 years – 119 (39.7%)
- more than 5 years – 80 (26.7%)

The research results were submitted to statistical analysis on the basis of the chi-squared test and Spearman's rank correlation. Results for which $p < 0,05$ were considered statistically significant.

Results

Table 1 presents occurrence of diabetes mellitus type 1 and 2 in subjects of two age groups. Diabetes mellitus type 2 was statistically significantly higher ($p < 0,05$) in patients over 40 years of age (98.16% of the subjects).

The duration of the disease was evaluated as well; the results are shown in table 2. The longer duration of the disease (more than 15 years) was noticed in patients with type 1 (37.35%) in comparison with patients with type 2 (22.58%).

The number of stays in a sanatorium is presented in table 3. The frequency of occurrence of diabetes mellitus among men and women was analyzed as well. It was ascertained that both men and women suffer from both types of diabetes at equal frequency. The place of residence – a city or a village – was not statistically significant. In the test group of 300 patients, 227 (75.67%) had, in various number, their own teeth. The state of hygiene was satisfactory, slightly worse in patients with a lower number of their own teeth but was not statistically significant.

The condition of the periodontium depending on the type of diabetes mellitus is shown in table 4.

A statistically significant interrelation between the condition of the periodontium and the type of diabetes mellitus was not observed. The level of compensation of diabetes and its duration had no statistically significant influence on the condition of the periodontium. It was stated, on the other hand, that in elderly patients, at the borderline of statistical significance ($p > 0,05$), the periodon-

Table 1. Occurrence of diabetes mellitus type 1 and 2 in subjects of two age groups

Subjects' age	Diabetes mellitus type		Total
	T1	T2	
Up to 39 years of age	29 (34.94 %)	4 (1.84 %)	33
From 40 years of age	54 (65.06 %)	213 (98.16 %)	267
Total	83	217	300
Pearson's Chi^2	67,17	df = 1	p = 0,00001
Spearman's R	0,47	t = 9,2724	p = 0,00001

Table 2. Duration of diabetes mellitus

Duration of diabetes mellitus	Diabetes mellitus type		Total
	T1	T2	
Up to 15 years	52 (62.65 %)	168 (77.42 %)	220
More than 15 years	31 (37.35 %)	49 (22.58 %)	80
Total	83	217	300

Table 3. Number of stays in a sanatorium

Number of stays in a sanatorium	Diabetes mellitus type		Total
	T1	T2	
1 x	20 (24.10 %)	76 (35.02 %)	96
2-5 x	33 (39.76 %)	106 (48.85 %)	139
> 5 x	30 (36.14 %)	35 (16.13 %)	65
Total	83	217	300

Table 4. The condition of the periodontium on the type of diabetes mellitus

Type of diabetes mellitus	Condition of the periodontium according to Bellini				Total
	B 0	B A	B B	B C	
Type 1	8 (11.27 %)	23 (32.39 %)	34 (47.89 %)	6 (8.45 %)	71
Type 2	8 (5.13 %)	53 (33.97 %)	70 (44.87 %)	25 (16.03 %)	156
Total	16	76	104	31	227
Pearson's Chi^2	4,79	df = 3	p = 0,18764		

tium required specialized treatment. The research also showed that treatment of the periodontium of patients rarely visiting sanatoria was statistically significantly ($p < 0,05$) more frequently needed.

Discussion

Increasing knowledge of basic development mechanisms and the course of diabetes mellitus allows designing new lines of treatment. While treating patients with diabetes mellitus, interdisciplinary action of a variety of specialists, including dentists, is imperative. The analysis of the test group confirmed more frequent occurrence of diabetes mellitus type 2 in elderly patients, which means that the risk of diabetes type 2 increases with age [1,5,8,12,15-17]. It was also ascertained that toothlessness occurred significantly more frequently in patients suffering from the disease for more than 15 years, with diabetes type 1 patients suffering from toothlessness considerably more often. Similar results were obtained by Dowey, who classified diabetes mellitus type 1 patients as the highest-risk group of patients vulnerable to a complete loss of teeth due to periodontal diseases [18]. Periodontal diseases in patients with diabetes mellitus require comprehensive prophylactic medical action. Influence of diabetes mellitus on periodontal diseases in highly controversial, there is more and more research on the subject. In the test group, a statistically significant connection between the condition of the periodontium and type of diabetes mellitus was not found. Some authors indicate such a connection between periodontal diseases and diabetes mellitus type 1 [10,19-24]. Other authors do not confirm the influence of diabetes mellitus on the condition of the periodontium [6,25-28]. Despite other author's reports [14,19,22,29-30] on an important correlation between the duration of diabetes and the stage of the development of periodontal diseases, no such correlation was noticed in the test group. Alpagot got similar results [25]. The aim of the research presented was to determine the influence of diabetes mellitus on the condition of the periodontium, and, consequently, may contribute to diabetologists taking more interest in the condition of the oral cavity of patients with diabetes mellitus.

Conclusion

No statistically significant interrelation between the condition of the periodontium, the level of compensation of diabetes mellitus and its duration was ascertained. In elderly patients, the periodontium more frequently required treatment.

No interrelation between the subjects' condition of the periodontium and the type of diabetes mellitus was observed. Men needed, at the borderline of statistical significance, treatment of the periodontium more frequently.

The number of stays in a sanatorium had a positive, statistically significant influence on the periodontium.

Toothlessness occurred significantly more often in patients suffering from diabetes mellitus for more than 15 years.

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Prevalence of malocclusion and orthodontic treatment need in Turkish adolescents

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Abstract

Objective: The objective of this study was to determine the prevalence of malocclusion and orthodontic treatment need in Turkish adolescents.

Method: The sample included 1125 boys and 1204 girls aged between 12 and 16 years. The dental health (DHC) and aesthetic (AC) components of the Index of Orthodontic Treatment Need (IOTN) were used as an assessment measure of the need for orthodontic treatment for the total sample.

Results: The results indicated a high prevalence of Class I (34.9 %) and Class II Division 1 malocclusions (40.0 %). Moreover, increased (18.3 %) and reduced bites (8.2 %), and increased (25.1 %) and reversed overjet (10.4 %) were present in the sample. Posterior crossbite was registered for 9.5 % whereas scissors bite was present in 0.3 % of the sample. Anterior crowding and midline diastema were detected in 65.1 and 7.0 % of the individuals, respectively.

Conclusion: Using the DHC of the IOTN, proportion of subjects estimated to have great and very great treatment need (grades 4 and 5) was 28.7 %. However, only 16.7 % of the individuals were in need (grades 8-10) of orthodontic treatment according to the AC. These results clearly indicated that Class II Division 1 malocclusion is the most prevalent occlusal pattern among the Turkish adolescents.

Key words: Index of Orthodontic Treatment Need, aesthetic, Turkish adolescents, prevalence of malocclusion

Introduction

On an increased basis, malocclusion is considered an expression of normal biologic variation, and treatment need is often based as much on psychosocial concerns as on proven oral health risks attributable to malocclusion (1). The criteria for determining who is most likely to benefit from orthodontic treatment are controversial. These factors make it particularly difficult for the general dentist to determine for whom orthodontic treatment is clearly indicated since the traditional pathway to orthodontic care starts at the general dentist's office.

Different populations have been investigated to provide epidemiological data of the prevalence of malocclusion (2-7). As a common trend, quantitative variables along with Angle's classification (1) were used in these reports. Additionally, treatment-need indexes were also used to determine orthodontic need based on esthetic impairment, potential for adverse effect on dental health, and deviation from normal occlusion. The Index of Orthodontic Treatment Need (IOTN), involving the Dental Health Component (DHC) and the Aesthetic Component (AC), is the most frequently used tool for measuring treatment need (8,9). Perhaps, being objective and synthetic, and allowing for comparisons between different population groups are the most important aspects of this index (7,10,11).

Certain European populations, such as Swedes (12), British (13), Germans (5,14), French(15), and Italian (6,7,16) have been examined extensively in regards to IOTN. Turkey, is an Eurasian country located in Western Asia (mostly in the Anatolianpeninsula) and in Southeastern Europe (East Thracian). Turkish population has genes from Asiatic Turks, Kurds, the Balkans, Caucasus, Middle East, Iran as well as from ancient Romans, Byzantines, and Arabs. There is little research and/or published data that evaluated the prevalence of malocclusion (17) and orthodontic treatment need (18) in Turkish individuals. Therefore, the aim of the present survey was to document the prevalence of individual traits of malocclusion, and to assess the need for orthodontic treatment in relation to gender by using the IOTN in a group of Turkish adolescent schoolchildren, and to compare the providing data with the findings of other European surveys.

Methods

Data was collected during an epidemiological survey in the period of May 2008 to December 2011 from 2329 adolescents (1125 males and 1204 females) aged 12,5–16,2 years randomly selected using a one-stage cluster sampling procedure in 13 state-funded secondary schools in Kirikkale city which is located in the south area of the capital. The schools were randomly selected from an initial pool of 27 schools that had been previously identified by the school district to avoid possible biases ensuing from social heterogeneity. Written parent informed consent forms were obtained for dental examinations. All male and female subjects who met the following criteria were included in the sample: (1) age 12 to 16 years, (2) secondary dentition present with no remaining deciduous teeth, (3) no multiple missing teeth, (4) presence of first permanent canines and molars, and (5) no previous history of orthodontic treatment. Each examination took place while the subject was seated in a standard quiet classroom in the designated chairs. The clinical examination was carried out by two examiners (IEG and AC), who were previously calibrated.

Orthodontic variables

Patients with an occlusal pattern that deviated from the ideal Class I relationship as described by Angle (1), (including crowding, spacing, rotations) were categorized as Class I malocclusion. Thus, the Class I normal category was limited to patients with occlusions that were ideal or near ideal. Patients with a different Angle classification of occlusion on each side were categorized into a single Class based on the predominant pattern of occlusion and/or canine relationship (4,19).

For overbite and overjet, values between 0 and 4 mm were considered normal (7). Posterior crossbite and scissors bite were registered as bilateral, right and left (4,5). Crowding was recorded for the incisor and also posterior segments of each jaw (1-3 mm= mild; 4-6 mm= moderate; >6 mm= severe) (4). Anterior diastema was diagnosed when there was a space of at least 1 mm between the central incisors in either arch (4).

Orthodontic treatment need

The findings served to determine "orthodontic treatment need" with reference to the IOTN (8,9) that consists of the DHC and the AC. Considerations as to "no treatment need", "borderline need", or "great need" were based on five grades in the DHC and 10 grades in the AC.

Statistical Analysis

To test and inter- and intra-examiner reproducibility, 25 children were re-examined 4 weeks after their initial examination using Kappa's method (20). The ratio of the sample, as a maximum estimate of the proportion of individual traits of malocclusion in the whole population was calculated for the total sample and for girls and boys separately. Number of subjects with diagnosed anomaly (n) and its prevalence (n/N x 100, where N is the number of subjects examined) was determined. The differences between gender groups were assessed by means of chi-square test. The data was analyzed with the SPSS software package (version 15.0, SPSS Inc., Chicago, III., USA) for IOTN DHC and AC grades. Level of significance was established at $p<0.05$.

Results

Kappa test indicated high reliability and reproducibility (kappa 0.73-0.80) for the parameters tested. Table 1 presents the prevalence of each occlusal trait in the total sample. Class I malocclusion was found in 812 subjects, which represented 34.9 % of the 2329 individuals examined. Class II malocclusion was diagnosed in 1041 individuals; 40.0 % of all patients were Division 1 and 4.7 % of all patients were Division 2. Class III malocclusion was found in 240 subjects 10.3 %. Normal overbite was the most common (73.5 %), mostly observed in girls ($P<0.001$). Increased overbite was recorded in 18.3 %, mostly observed in boys ($P<0.05$). The prevalence of reduced bite value was found as 8.2 %. Normal overjet was present in 1501 individuals (64.5 %). Prevalence of increased overjet (25.1 %) was found to be higher than negative overjet (10.4 %). While bilateral crossbite was found more frequently as much as of 4.0 % of the sample, scissors bite was rare being diagnosed in only 0.3 percent of the subjects.

Anterior crowding was present in 1638 individuals (65.1 %) (Table 2); 17.9, 9.1 and 38.1 % of those had crowding in the upper arch, the

Table 1. Occlusal classifications

			Boys		Girls		Total		P	
			n	%	n	%	n	%		
Occlusal anteroposterior relationships										
Normal Occlusion			110	9.8	126	10.5	236	10.1	NS	0.63
Class I			404	35.9	408	33.9	812	34.9	NS	0.317
Class II Division 1			448	39.8	483	40.1	931	40.0	NS	0.899
Class II Division 2			56	5.0	54	4.5	110	4.7	NS	0.625
Class III			107	9.5	133	11.0	240	10.3	NS	0.246
Distribution of overbite										
Normal, 0-4 mm			802	71.2	913	75.8	1715	73.5	***	0.0001
Increased, > 4 mm			227	20.2	197	16.4	424	18.3	*	0.018
Reduced, < 0 mm			96	8.5	94	7.8	190	8.2	NS	0.098
Distribution of overjet										
Normal			731	65	770	64	1501	64.5	NS	0.866
Increased			281	25	304	25.2	585	25.1	NS	0.886
Negative			113	10	130	10.8	243	10.4	NS	0.588
Distribution of posterior crossbite and scissors bite										
No finding			1021	90.8	1082	89.9	2103	90.3	NS	0.677
Crossbite	Bilateral		41	3.6	52	4.3	93	4.0	NS	0.544
	Unilateral	right	35	3.1	41	3.4	76	3.3	NS	0.890
		left	24	2.1	27	2.2	51	2.2	NS	0.970
Scissors bite	Bilateral		1	0.1	1	0.1	2	0.1	NS	0.957
	Unilateral	right	2	0.2	0	0.0	2	0.1	NS	0.949
		left	1	0.1	1	0.1	2	0.1	NS	0.889

*NS. Not significant. *P<.05; ***P<.001.*

Table 2. Distribution of crowding and diastema

n		Boys		Girls		Total		P	
		%	n	%	n	%			
Crowding									
No crowding		383	34.0	428	35.5	811	34.8	NS	0.460
Only Upper arch	mild	140	12.4	120	10.0	260	11.2	NS	0.214
	moderate	55	4.9	60	5.0	115	4.9	NS	0.732
	severe	18	1.6	24	2.0	42	1.8	NS	0.810
Only Lower arch	mild	67	6.0	70	5.8	137	5.9	NS	0.845
	moderate	28	2.5	31	2.6	59	2.5	NS	0.760
	severe	8	0.7	9	0.7	17	0.7	NS	0.77
Both arches	mild	280	24.9	303	25.2	583	25.0	NS	0.981
	moderate	127	11.3	137	11.4	264	11.3	NS	0.831
	severe	19	1.7	22	1.8	41	1.8	NS	0.985
Diastema									
No finding		808	71.8	852	70.8	1660	71.3	NS	0.328
Upper arch	midline	140	12.4	156	13	296	12.7	NS	0.214
	spread	99	8.8	95	7.9	194	8.4	NS	0.632
Lower arch	midline	20	1.8	30	2.4	50	2.1	NS	0.870
	spread	58	5.2	71	5.9	129	5.5	NS	0.670

NS. Not significant.

lower arch and both arches, respectively. Moderate crowding was more common in both arches. Midline and spread diastemas were found in 14.8 and 13.9 % of the sample, respectively. Diastemas were observed mostly in the upper arch (Table 2).

In the study group, the IOTN revealed "no treatment need" in 45.6 % using the DHC (mostly in boys (P<.05)) and 43.1 % using the AC. When borderline cases were taken into consideration, the treatment need was diagnosed in 25.7 % with the DHC and in 40.2 % with the AC. The number of subjects with the need for orthodontic treatment was 648 (28.7 %) using the DHC, and 376 (16.7 %) with the AC (Figures 1, 2 and Tables 3, 4).

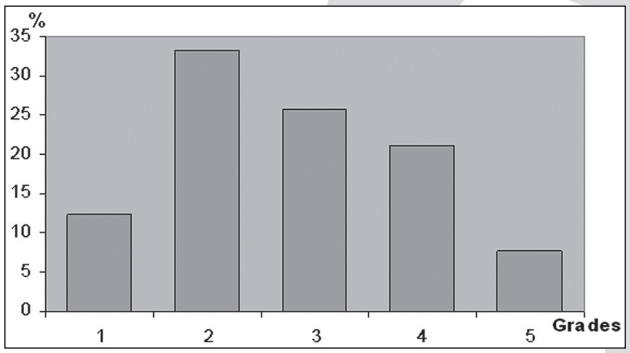

Figure 1. Dental health component (DHC) grades of the Index of Orthodontic Treatment Need in Turkish adolescents (Grades 1 and 2, 'no need'; Grade 3, 'borderline need'; Grades 4 and 5, 'definite need')

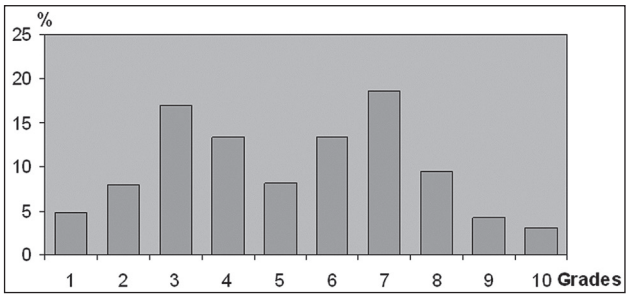

Figure 2. Aesthetic component (AC) grades of the Index of Orthodontic Treatment Need in Turkish adolescents. (Grades 1-4, 'no need'; Grade 5-7, 'borderline need'; Grades 8-10, 'definite need')

Discussion

Although many studies were published to describe the prevalence and types of malocclusion, when examining a certain population it is difficult to compare and contrast these findings, partly because of the varying methods and indices used to assess and record occlusal relationships, age differences of the study populations, examiner subjectivity, specific objectives, and differing sample sizes (19). Methodology used in this study was mostly collected from European studies (4,6,7,19), and our results were discussed with the findings from different European geological regions because of the close proximity and since there was limited information on Turkish individuals in the literature. The general consensus about treatment timing for malocclusions is that it should start around perma-

Table 3. The DHC of IOTN statistics of boys and girls

Occlusal anteroposterior relationships	Boys		Girls		Total		P	
	n	%	n	%	n	%		
No Need	531	48.3	492	42.8	1023	45.6	*	0.01
Borderline Need	263	23.9	316	27.5	579	25.7	NS	0.054
Need	306	27.8	342	29.7	648	28.7	NS	0.328
Total	1100	100	1150	100	2250	100		

**, P<.05; NS, Not significant.*

Table 4. The AC of IOTN statistics of boys and girls

Occlusal anteroposterior relationships	Boys		Girls		Total		P	
	n	%	n	%	n	%		
No Need	492	44.6	478	41.6	970	43.1	NS	0.136
Borderline Need	437	39.6	467	40.7	904	40.2	NS	0.699
Need	171	15.4	205	17.9	376	16.7	NS	0.142
Total	1100	100	1150	100	2250	100		

NS, Not significant.

nent dentition. At this stage maxillary and mandibular development is almost completed and the malocclusion takes its final pattern. Given the characteristics of the sample, this paper demonstrated the occlusal traits of an untreated adolescent population at those ages.

With respect to the occlusal findings, Class I malocclusion was found in 34.9 % of the sample. This Class I occlusion figure included individuals with incisor crowding and dental malalignment and thus did not imply ideal Class I occlusion. The prevalence of Class II Division 1 (40.0 %) in the present study was greater than the rates reported by Haynes (21) (for English school children, 12.5 %), Foster and Day (22) (in a Shrophire school population, 27.2 %), Thilander et al.(4) (adolescents in Bogotá, 14.9 %), Perillo et al.(7) (for Italian school adolescents, 36.3 %) and Celikoglu et al.(23) (for Turkish patient, 28.9 %). However, Lauc (24) (on Hvar Island) and Josefsson et al.(12) (for a Swedish population) found that Class II malocclusion was more common in their population (greater than 45 percent), and explained this figure by a genetic influence on the incidence of Class II malocclusions. Early treatment in the primary or early mixed dentition has been recommended for Class III malocclusions (4). The prevalence of Class III malocclusion determined in this study is 10.3 %. However, Goose et al.(25) (2.91 %), Haynes (21) (2.5 %), Foster and Day (22) (3.5 %), Proffit et al.(26) (5.7 %), Thilander et al.(4) (5.8 %), Lauc (24) (4.8 %) and, Perillo et al.(7) (4.3 %) reported lower rates. The present study confirmed that the predominant anteroposterior relationship of the arches in Turkish adolescents was Class II Division 1. Of the vertical anomalies, increased overbite was more than twice as frequent as anterior open bite. Our results were similar to the rates reported by Thilander et al.(4) and Lauc (24) who also said a deep bite was often associated with a Class II malocclusion and more common in boys. However it was seen higher ratios in Italian samples (6,7). Increased overjet showed high percentage as increased overbite in this study; this is a reflection of the higher prevalence of Class II malocclusion among Turkish adolescents. Our findings agree with those of Thilander et al.(4) in Bogotanian adolescents and Ciuffolo et al.(6) in Italian adolescents where high rates of increased overjet in the permanent dentition were reported. A France sample (3), increased overjet was showed present in fewer subjects (6 %).

In this study, uni/bilateral posterior crossbite was more frequent than scissor bite and was observed in 9.5 % of the sample. This rate was similar to the findings of Ciuffolo et al.(6) was higher than Thilander et al.(4) Perillo et al.(7) showed some higher % for crossbite and scissor bite (14.2 and 3.5 %, respectively).

Crowding in one or both arches was the most frequent of all anomalies recorded (65.1 %). This finding complied with the results of Thilander et al.(4) and Lauc (24). There is a general consensus that treatment of crowding should start in the permanent dentition (5). The National Health and Nutrition Survey III undertaken in the United States between 1989 and 1994 showed a frequency of crowding ranging from 42.3 % at ages 8–11 to 54.5 % at ages 12–17 (26) which was lower than the frequencies observed in this investigation. Nevertheless, other studies have reported lower rates of crowding with located in anterior/both segment (3,6,7,21,22).

Thilander et al.(4) found the prevalence of median diastema in their population to be 13.5 % in the early mixed and 4 percent in the permanent dentition. Lauc (24) observed a high rate of midline diastema (45.1 %). In contrast, this rate for our study was 12.7 %. Perillo et al.(7) showed the prevalence of median diastema as 9.9 percent. The frequency of diastema in Nigeria was 24 % (27). Onyeaso (28) indicated that diastema is not regarded as a malocclusion among Nigerians but as a mark of natural beauty.

Administrators of publicly funded programs need a valid screening method for determining priority for orthodontic treatment. Priority of orthodontic care through national health care plans in European countries has been a prime factor behind the development of indexes such as the IOTN.

The need for orthodontic treatment has been presented in the literature using different indices. In the present study, the classification by the IOTN was used as the authors are familiar with this index.

In Turkey, there are few epidemiologic surveys. Ugur et al.(18) were found 37.77 % of orthodontic treatment need using the TPI in 6–10-year-old 572 Turkish primary school children with a high socio-

economic standard in central Anatolia. Our study was carried out in wide adolescent sample that has moderate socioeconomic status and the treatment need was lower than Ugur et al.'s study. So the results of this study were not in agreement with Ugur et al.(18) who determined that the orthodontic treatment needs increase with age. Hamamci et al.(29) used the Dental Aesthetic Index (DAI) measured awareness of malocclusion between Turkish university students. Dogan et al.(30) used the DHC and the AC of IOTN in 9–18-year-old 208 Turkish individuals and, found 74.0 and 51.4 % of orthodontic treatment need respectively. The latter two studies were carried out using the same age group, strict standardization protocol and had less sample size.

In this study according to the DHC of the IOTN, 28.7 percent of the whole sample was classified as being in need of orthodontic treatment (grades 4 and 5). The results show that the percentage is relatively greater than those reported by Souames et al.(15) in France and Perillo et al.(7) in Italy (21.3 and 27.3 % respectively). However, the British studies found a higher prevalence rate for untreated subjects: 32.7 % (9) and, 35 % (13). Josefsson et al.(12) found 39.5 % of orthodontic treatment need in a Swedish sample. The findings of the present study, therefore, indicate that a substantial need for orthodontic intervention was present at a similar level to French and Italian children but generally lower than northern European populations (United Kingdom and Sweden).

The AC component for IOTN in the present study reduced the orthodontic treatment need (16.7 %). This has also been reported in other studies (9,12,15). Tausche et al.(5) said the AC alone failed to identify any children needing orthodontic treatment. Hovewer, Josefsson et al.(12) used the AC both by the examiner and the subject. This study also hunts up a difference between genders for orthodontic treatment need. The treatment need did not differ significantly as a result of gender.

Conclusion

The results of this investigation demonstrated that Class II Division 1 malocclusion is the most prevalent occlusal pattern among Turkish adolescents and the high incidence of increased overjet and overbite are a reflection of the high prevalence of Class II malocclusion. Also a high percentage of the crowding is noteworthy. Almost one- third of the evaluated population would have a mandatory need for orthodontic treatment, if the DHC scores were used as the main criterion for such decisions. If the AC scores were used, the need would decrease to one-fifth of the sample.

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Efficacy and safety of exenatide in obese type 2 diabetic patients of poor glycemic control with oral therapy

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Abstract

Aim: To compared the efficacy and safety of exenatide and glargine in obese type 2 diabetes of poor glycemic control with oral antidiabetic drugs(OAD) therapy.

Methods: 44 Patients were randomly initiated exenatide (n=20) or glargine (n=22) based treatment and finically completed the 24-week study. We recorded the data (HbA1C, FPG, 2hPG, body weight, waist circumstance, hip circumstance) at baseline and the end of study. The data of hypoglycemia rate and adverse events during the treatment were also collected. BMI and WHR were calculated.

Results: At 24 weeks, A1c decreased from baseline (7.9%) to 6.7% in exenatide group and 7.8% to 6.8% in glargine group without difference. The decrease of FPG in two groups was -2.6mmol/L and -3.3mmol/L, respectively. Mean 2h PG decreased from 13.5mmol/L to 8.9mmol/L in exenatide group vs. 13.2mmol/L to 9.9mmol/L in glargine group (P<0.01). HOMA-IR, AUC-INS and HOMA-βimproved in both groups with a significant difference from baseline (P values all <0.05). But there was no obvious difference between groups. Patients given exenatide injection achieved a remarkable decrease in body weight, BMI, waist circumstance and WHR (P<0.01) with a dosage of 10ug per day, while patients in glargine group obtained an increase changes in these parameters (P<0.01) with a dosage of 0.38±0.04U/kg /day. Hypoglycemia rate was lower in exenatide group compared to glargine group (P<0.01).

Conclusions: Exenatide and glargine were effective in obese type 2 diabetes who were unable to achieve adequate glycemic control with OAD therapy. Exenatide didn't increase the hypoglycemia risk and shown a significant advantage in weight loss. The gastrointestinal-related adverse events were the most common AEs which were transient.

Key words: Obese type 2 diabetes, GLP-1, exenatide, glargine, safety.

Introduction

Glycemic control is still a challenge for the obese type 2 diabetic patients which are difficult to reach the treatment goal by oral antidiabetic drugs (OAD) therapy. Exenatide is a new class agent of incretin peptide mimetics (glucagon-like polypeptide-1 [GLP-1] receptor agonists) which decreases the blood glucose by suppressing glucagon secretion, promoting insulin synthesis and secretion and decreasing gastric emptying. The aim of this study was to assess the efficacy and safety of exenatide in obese type 2 diabetes patients of poor blood glucose control with oral antidiabetic drugs.

Subjects and Methods

Subjects

42 patients from our hospital during November 2009 to May 2010 were included in our study. All of them were obese type 2 diabetic patients unable to achieve adequate glycemic control with OAD therapy. Inclusion criteria included: 1. Presence of type 2 diabetes (according to the 1999 WHO criteria for the diagnosis of diabetes mellitus); 2. Obesity (waist-to-hip ratio (WHR) above 0.85 for female and 0.9 for male or body mass index (BMI) ≥25kg/m^2, according to 2000 WHO Asia-Pacific Region recommended); 3. Taking one or two kinds of oral antidiabetic drugs with a dosage at least 50% of maximum recommended doses for more than three months and fasting blood glucose (FBG) was still above 7.0mmol/L and HbA1c was above 7.5%. Exclusion criteria included type 1 diabetes mellitus, infectious disease, tumor, pancreatic disease, heart problems, liver disease and recent happened acute diabetic complications.

Methods

All patients provided written informed consent before participation in their respective clinical trials. 42 Patients were randomly assigned into exenatide group (20) and glargine group (22). In exenatide group, initial 5ug exenatide injections were added to the previous OAD therapy by twice daily 30-60 min before morning and evening meals for the first month and if without hypoglycemia, increased to 10ug twice daily since the second month. In glargine group, in addition to the OAD therapy, glargine injections were given before bedtime. The initial dose was 0.2u/kg/d or 10 U/d and then gradually titrated until fast plasma glucose (FPG) ≤6.0mmol/L.

Height, body weight, waist circumstance, hip circumstance, blood pressure, BMI, WHR were recorded at baseline and weeks 24. Liver and renal function, blood lipid, oral glucose tolerance test (OGTT) and insulin release test (IRT) were also tested at weeks 0 and 24. Homeostasis model assessment of beta-cell function (HOMA- β), insulin resistance index (HOME-IR) and the area under insulin curve (AUC-INS) were calculated at weeks 24. Clinical blood samples were analyzed by clinical laboratories using standard methods. Serum insulin was quantitated by chemiluminescent immunoassay. HbA1C was measured using high-performance liquid chromatography.

Statistical analysis

All statistical analyses were analyzed by SPSS 17.0 for Windows (SPSS Inc, Chicago, IL). Data with non-normal distribution (AUC-INS, HOMA-IR and HOMA-β) were analyzed after the logarithmic transformation. Quantitative data were presented as mean ± SD. The significance of the differences between means was assessed by T test. A P value <0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 20 patients were enrolled in Byetta group(male 14, female 6) and 22 patients(male 14, female 8) in Lastus group. Patient disposition is shown in Table 1. There was no significant difference between two groups in general data at baseline.

Table 1. Subject's clinical and biological characteristics at baseline ($\bar{x}\pm s$)

Group	Exenatide	Glargine
Male/female	20(14/6)	22(14/8)
Age(y)	48±7.2	49.0±6.5
Duration(y)	6.7±3.1	6.4±3.7
BMI(kg/m^2)	28.2±2.59	27.6±2.62
FBS(mmol/L)	9.7±1.9	9.5±1.8
2H(mmol/L)	13.5±2.5	13.2±2.1
GHbA1c%	7.9±1.0	7.8±0.9
AST(u/L)	23±2.16	20±2.13
ALT(u/L)	15±1.6	19±1.8
Cr(ummol/L)	81.1±5.4	83.3±5.5
Alb/cr(mg/g)	13±2	14±2
TC(mmol/L)	5.87±1.09	4.97±0.54
TG(mmol/L)	3.73±0.54	3.07±0.89
HDL(mmol/L)	1.36±0.23	1.32±0.24
LDL(mmol/L)	3.46±0.86	3.61±0.45
SBP(mmHg)	132±12	133±10
DBP(mmHg)	82±10	81±11

Changes in FPG, 2hPG, HbA1c, HOMA-IR, HOMA-β and AUC-INS after treatment

The changes of FPG, 2hPG, HbA1c, HOMA-IR, HOMA-β and AUC-INS were shown in Table 2. At 24 weeks, HbA1c decreased from baseline by 1.2% for exenatide compared to 1.0% for glargine. And the decreases of FPG in two groups were -2.6mmol/L and -3.3mmol/L, respectively. Mean 2h PG decreased from 13.5mmol/L to 8.9mmol/L in exenatide group, while in glargine group it decreased from 13.2mmol/L to 9.9mmol/L. HOMA-IR and AUC-INS were significantly declined after treatment in both groups (p=0.025 in exenatide group and p=0.028 in glargine group). HOMA-β was significantly higher in both groups compared with the baseline (p=0.036 in exenatide group and p=0.031 in glargine group). There was no significant difference between two groups in these indexes. After the 24-week treatment, TG, CHO-C and LDL-C were significant lower than before (p<0.05). We found no statistically difference in HDL, SBP, DBP and urinary albumin-to-creatinine ratio.

Changes in weight, BMI, waist circumstance and WHR

Changes in weight, BMI, waist circumstance and WHR between groups were shown in Table 3. Comparison with the baseline characteristics,

Table 2. Changes between exenatide and glargine group before and after 6-month treatment ($\bar{x}\pm s$)

Group		FPG (mmol/l)	2hPG (mmol/l)	HbA1c (%)	Ln(HOMR-IR)	Ln(HOMR-β)	AUC-INS
Exenatide	Before	9.7±1.9	13.5±2.5	7.9±1.0	6.3±1.8	40.83±4.25	0.64±0.35
	After	7.1±1.7*	8.9±2.2*Δ	6.7±0.8*	2.8±1.5*	78.27±8.37*	0.35±0.21*
Glargine	Before	9.5±1.8	13.2±2.1	7.8±0.9	6.5±1.9	38.65±4.04	0.73±0.42
	After	6.2±1.5*Δ	9.9±2.0*	6.8±0.9*	3.0±1.7*	84.17±9.87*	0.46±0.27*

** compared with baseline, P<0.05.*
Δ compared with Exenatide group, P< 0.05.
a HOMA-IR= I0×G/22.5, G=glucose, I=insulin.
b HOMR-β= I0×20/(G-3.5) , G=glucose, I=insulin.
c AUC-INS=I0+I180/2+I30+I60+I120, G=glucose, I=insulin.

Table 3. Changes in weight, BMI, waist circumstance and WHR between groups ($\bar{x}\pm s$)

Group		Weight (kg)	BMI(kg/m^2)	Waist (cm)	WHR
Exenatide	Before	86.3±10.9	28.2±2.5	94.7±10.7	0.95±0.06
	After	82.8±11.0*	27.1±2.4*	89.2±9.7*	0.91±0.07*
Glargine	Before	85.6±10.2	27.6±2.6	93.3±9.4	0.94±0.04
	After	88.1±10.4Δ*	29.2±2.2Δ*	95.6±9.8Δ*	0.96±0.09Δ*

** compared with baseline, P<0.05.*
Δ compared with Exenatide group, P< 0.05.

patients in exenatide group showed an advantage in weight loss. At the end point of the study, weight, BMI, waist circumference and WHR in exenatide 10ug group were decreased 3.5kg, 1.1kg/m^2, 5.5cm, 4% from baseline, respectively (P<0.01). Conversely, the changes from baseline were +2.5kg, +1.6kg/m^2, +2.3cm and +2% respectively in Glargine group, increased significantly (p<0.01). The average dose of glargine was 27.6±1.2U/d or 0.38±0.04U/kg/d.

Incidence of hypoglycemia and other adverse effects

The hypoglycemia rate in exenatide group was 0.1 person-time, without nocturnal hypoglycemia occurred, compared with 0.18 and 0.045 person-time in glargine group. The difference between groups was statistically significant (p<0.05). In exenatide group, 6 patients (30%) experienced mild nausea, bloating and dyspepsia during the first-month therapy. 7 patients (35%) had the similar experience during the transformation from 5ug to 10ug in the second-month therapy. But all the adverse events were temporary. 1 patient in exenatide group had discontinued treatment because of gastrointestinal reaction during the second month (after administration of 10ug for 5 days). No patients in glargine group reported any gastrointestinal adverse events.

Discussion

It is usually difficult for type 2 diabetes patients with obesity to reach glycemic control target. In these patients, hyperglycemia always dues to excessive calorie intake and leads to increasing dose of antidiabetic agents. Hypoglycemia may occur more frequent after inappropriate dose adjustment. Fear of hypoglycemia makes them eat a lot. This induces weight gaining and metabolic disorders deterioration. All of these risk factors will contribute to the progression of diabetes and complications [1, 2]. The conventional therapy for those obese type 2 diabetic patients was adding premixed or basal insulin to their original OAD treatment. However, the risk of hypoglycemia and weight gain was increasing [3]. Exenatide is the first drug of the incretin mimetic class. The glucoregulatory activities include enhancing glucose-dependent insulin synthesis and secretion from pancreatic beta cells, decreasing glucagon production, appetite suppression, slowing gastric emptying time, weight loss and decreasing blood glucose [4]. Glargine is long-acting insulin that can simulate the secretion of basal insulin and also has an advantage in fasting and postprandial blood glucose control. The compliance to once-daily administration was proved to be good. We observed a similar effect between exenatide and glargine in

glycemic control. Patients in both groups achieved significant decrease in FBG、2PG and HbA1c. However exenatide had advantage in lowing postprandial blood glucose compared to glargine and was disadvantage in lowing FPG, as Kendall DM reported before [5]. After the 24-week treatment, TG, CHO-C and LDL-C were significant lower than baseline, HDL-c trended to be elevated and blood pressure improved, but there was no significant difference between two therapies. Improvement in glucose metabolism was considered to be associated with the changes in lipids and blood pressure. These treatments had no influence on liver function, renal function and urinary albumin-to-creatinine ratio, which was similar to the report before [6].

Obesity is an important risk factor for type 2 diabetes. The results of our study showed a difference in weight changing between groups after 24-week treatment. Patients in exenatide group decreased 3.46kg in weight and 4% in WHR. These changes contributed to the improvement of insulin secretion and sensitivity. Patients in glargine group gained weight during the treatment period. It was thought to be associated with the synthesis and metabolism of insulin.

Hypoglycemia is an obstacle factor for obese type 2 diabetic patients to achieve glucose control. In our study, 2 patients in exenatide group experienced hypoglycemia when treated with a dosage of 10ug bid (blood glucose=3.9mmol/L and 4.5mmol/L, respectively). 4 patients in glargine group reported hypoglycemia events with one case of which was nocturnal hypoglycemia. Hypoglycemia rate was lower in exenatide group, mainly because of its glucose-dependent secretion of insulin [6]. However, we should mention that patients in exenatide group reported more gastrointestinal-related adverse events than those in glargine group. The most common AEs were mild nausea and diarrhea. These AEs always occurred during the first-week treatment. AEs cases reported by women were much more than those from men. The mechanism deserves to further research.

In conclusion, both exenatide and glargine provided good effect on glycemic control in obese type 2 diabetic patients who were unable to achieve adequate glycemic control with OAD therapy. Clinical physicians are suggested to provide individualized treatment regarding patients' characteristics. For those who concern on hypoglycemia and weight gain, it is recommended to choose exenatide as their first choice.

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Fatal complication, rescue therapy; covered stent for coronary artery perforation

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Abstract

Although coronary perforation is a rare complication observed during interventional procedures, it has a considerably high mortality rate. The prevelance of coronary perforation has been reported to be 0,20-0,6% (1-4). Its sudden development, the patient's agitation and development of rapid collapse renders intervention difficult. The presence of perfusion balloon and covered stent in clinics are life-saving. In the present study, we retrospectively reviewed 17 cases with coronary artery perforation who were treated between 2009 and 2012. Of these patients, 10 (58,8%) were men and 7 (41,2%) were women; the median age was 62,8±8,3 years. The coronary artery perforation resulted from guide wire in 23,5%, balloon dilatation in 58,8% and stent implantation in 17,6%. All the lesions were either type B or C lesions. The extensiveness of perforation was Ellis grade I in 23,5%, grade II in 47,1% and grade III in 29,4% of the cases. In the treatment of the perforation, polytetrafluoroethylene-covered stent graft was implemented in 9 (52,9%) patients, whilst conventional and emergency surgical therapy was performed in 8 (47,1%) patients. Grade I perforations occurred due to the guide wire and were managed with conventional therapy ($p<0,05$). Grade II and III perforations resulted from balloon and stent. The majority of these patients were inserted Graft Stent (stent graft in 52,9% and conventional therapy in 23,5% of the cases ($p<0,05$). Although all the stent grafts were successfully implanted, the complete control of bleeding was achieved only in 77,7% of the patients. Mortality was not observed in grade I perforation, whilst all cases resulted in mortality had grade III perforation.

These data indicate that there is a need for further advanced technology in the coronary artery perforation despite of currently available therapeutic options.

Key words: Percutaneous coronary artery intervention, Coronary artery perforation, Covered stent.

Introduction

Coronary artery perforation is an emergency situation which is encountered rarely. Its incidence was reported to be 0,2-0,6%.[1,4] In our country, this situation was reported only as case reports in the medical literature, but there was no prospective study about this issue.[5,6] Its acute onset, and rapid progression to collapse and tamponade make interventions difficult. The interventions performed for type B and C lesions defined in AHA/ACC, and atheroablation procedures that are increasingly practiced have increased the frequency of the occurrence of perforation. It has been demonstrated that the type of the guide wire used, the balloon/vascular diameter ratio, and oversize stent are the factors for the development of perforation. The choice of large balloon, and high inflation pressure may result in perforation.[1,3,7,13] It is life-saving that the cardiologist performing the invasive procedure is competent and aware of this complication, avoids this complication and has accurate knowledge about how should act when this complication occurs.[11] The coronary ruptures which have formed due to invasive procedure interventions are treated according to rupture types –the first groups are those treated by greft stent and the other group is those that are treated conservatively. The aim of this study is to compare these two groups –namely those ruptures treated by greft stent and those that are treated conservatively.

Materials and method

In this study, 7452 patients who underwent percutaneous coronary artery interventions in the period between 2009 and 2012 were retrospectively

reviewed by two experienced cardiologist for coronary artery perforations. The cineangiograms (Axiom Artis, Simens, Germany) of 17 patients with perforations were evaluated. Clinical and demographic features of the patients, the vessels in which the procedure was performed, the type of lesion, the cause of perforation and the extensiveness of perforation were recorded. The patients were divided into two groups depending on the treatment modalities implemented after perforation. The group I consisted of the cases treated with polytetrafluoroethylene (PTFE)-covered stent and the group II consisted of the patients treated with conventional approaches. The AHA/ACC classification was used to determine the type of lesion. Ellis grading system was used for the largeness of perforation. Ellis grade I was defined as the formation of extra-luminal crater; grade II as pericardial or myocardial blush without contrast jetting; an grade III was defined as perforations larger than 1 mm in diameter. This study protocol was approved by ethics committee of hospital and all patients' relatives gave their informed consent.

Statistical analysis

For statistical analyses, SPSS version 17 pack program (SPSS Inc., Chicago, Illinois, USA) was used. In the analyses of differences, chi-square analysis for appropriate chi-square distribution parameters, and Mann Whitney U test were used. Any p value <0,05 was set to be significant.

Results

Of the patients, 10 (58,8%) were men and 7 (41,2%) were women; the median age of the patients was 62,8±8,3 years. Of the vessels in which the intervention was done, 11 (64,7%) were LAD and 6 (35,3%) were CX. All coronary artery lesions were type B and C lesions according to AHA/ACC classification. There was no statistically significant difference between two groups with regard to demographic features and perforated vessel. Given the causes of perforation, it was found that guide wire-related perforations were grade I lesions and the perforations due to the balloon and stent were grade II or grade III perforations. This situation was found to be significant (p<0,05) (Table 1).

Among the patients in the group I (covered stent group), the causes of perforation were balloon (66,7%) and stent (33,3%). In the group II (conventional group) patients, the causes of perforation were found to be guide wire (50%) and balloon (50%). The differences between the groups were found to be significant (p<0,05). By the largeness of perforation, the frequencies of grade I, II, and III perforations were found to be 0%, 55,6%, and 44,4%, respectively, in the group I and 50%, 37,5%, and 12,5%, respectively, in the group II. The differences were statistically significant (Table 2).

The grade I perforation was not observed in the group I patients, whilst the grade III perforation was found in only one patient in the group II. When

Table 1. The relation between Ellis grade and perforation causes

Parameters	Group I (n=9)		Group II (n=8)		Total (n=17)		*p*
	n	%	n	%	n	%	
Grade I							
Guide wire	0	00.0	4	100.0	4	100.0	**<0.05**
Balloon	0	00.0	0	00.0	0	00.0	
Stent	0	00.0	0	00.0	0	00.0	
Grade II							
Guide wire	0	00.0	0	00.0	0	00.0	**<0.05**
Balloon	3	60.0	3	100.0	6	75.0	
Stent	2	40.0	0	00.0	2	25.0	
Grade III							
Guide wire	0	00.0	0	00.0	0	00.0	**<0.05**
Balloon	3	75.0	1	100.0	4	80.0	
Stent	1	25.0	0	00.0	1	20.0	

Group I: Covered Stent; Group II: Conventional Treatment; Grade: Ellis Grade

Table 2. Angiographic, demographic and clinic characteristics of the patients

Parameters	Group I (n=9)		Group II (n=8)		Total (n=17)		*p**
	n	%	n	%	n	%	
Gender							
Male	5	55.6	5	62.5	10	58.8	0.815
Female	4	44.4	3	37.5	7	41.2	
Risk Factor							
DM	4	44.4	4	50.0	8	47.0	0.139
HT	3	33.3	1	12.5	4	23.5	
HL	2	22.2	1	12.5	3	17.7	
Lesioned Vessel							
LAD	7	77.8	4	50.0	11	64.7	0.370
CX	2	22.2	4	50.0	6	35.3	
Lesion Type							
Type B	6	66.7	3	37.5	9	52.9	0.321
Type C	3	33.3	5	62.5	8	47.1	
Mechanism of Rupture							
Guide wire	0	0	4	50.0	4	23.5	**<0,05**
Balloon	6	66.7	4	50.0	10	58.8	
Stent	3	33.3	0	0	3	17.6	
Perforation Severity							
Grade I	0	0	4	50.0	4	23.5	**0.036***
Grade II	5	55.6	3	37.5	8	47.1	
Grade III	4	44.4	1	12.5	5	29.4	

** Test result is significant at 0,05 level (%95 C.I.) * Mann Whitney-U Test; Group I: Covered Stent; Group II: Conventional Treatment, Grade: Ellis Grade. Lesion type: AHA/ACC classification for coronary lesion, DM: Diabetes Mellutus; HT: Hypertension; HL: Hyperlypidemia*

all patients were evaluated, 58,8% of the patients were men, 47% were diabetics. It was observed that the perforation occurred in LAD (64,7%), type B lesions (52,9%) and 47,1% of perforations were grade II perforations. In the group I, although all stent grafts were successfully implanted, complete control of bleeding was achieved in 77,7% of the patients. In the group II, medical treatment and follow up, prolonged balloon inflation, pericardiocentesis, and/or emergency surgical treatment were performed. The in-hospital mortality rate was calculated as 11,7% for whole patients.

Treatment Options

Covered stent (Group I): The polytetrafluoroethylene-covered JoStent Graftmaster VR Stent (Abbott Vascular, Redwood city, CA, USA) was inserted in 9 (52,9%) of 17 patients because of Ellis grade II and III perforations. The patients were successfully implanted JoStent Graftmaster (sizes 3×16 mm or 3.5×19 mm) stents with a pressure of 16-18 atmosphere. One stent sufficed in all cases. After the procedure, thrombolysis in myocardial infarction 3 (TIMI 3) flow and full control of bleeding (77,7%) were ensured. Postoperative mortality occurred in two patients with grade III perforation, in whom stent graft couldn't suffice to control bleeding and therefore emergency surgery was performed.

Conventional treatment (Group II): Conventional treatment seems to be the treatment of choice for grade I and II perforations. In our study, 8 cases were treated in conventional manner. Conventional treatment involves medical treatment, balloon tamponade, pericardiocentesis and surgical treatment.

Medical treatment: Once the diagnosis of perforation was made, it was given intravenously 1mg of protamine for every 100 units of heparin. If hypotension developed, physiological saline and vasopressor infusion (dopamine, 10-15 microgram/kg/min) were given; the patients who developed vagal reaction, 0,5-1 mg of atropine was administered.

Balloon tamponade: In grade I and II perforations, compression was made inflating the balloon of stent used with low-pressure proximally to or within the perforation area. A perfusion balloon was used in case there was a need for prolonged balloon inflation.

Pericardiocentesis and surgical treatment: The cases who developed tamponade were first performed pericardiocentesis. Surgical repair was done if the symptoms of tamponade did not resolve with pericardiocentesis, infusions of fluid and vasopressors, and consequently cardiogenic shock emerged. Surgical intervention resulted in mortality in 2 of 5 patients. The rate of surgical mortality was 2/5 (40%). Of the patients having grade I perforations, one case was performed surgical intervention since there were signs of tamponade 7 hours after pci, other one patient was performed pericardiocentesis alone. Mortality was not observed among the cases with grade I perforations.

Discussion

In our study in the ellis type 2-3 rupture caused by invasive procedures, greft stent was implanted with a success rate of 50 % and the rupture was taken under control 77.7%. In cases in which greft stent could not be implanted and in cases where the stent could not treat the rupture, the mortality rate among the patients who were operated has reached 40%.

Since the percutaneous coronary angioplasty was first performed by Andreass Gruetzing in humans in 1977, it has begun to perform interventions for more complicated coronary lesions in parallel to the technological advances. The significance of coronary artery perforation is resulted from the sudden emergence of its clinical picture and high mortality rate. Therefore, it is important to previously recognize the cases at risk. The clinical risk factors involve advanced age, cardiac failure, low creatinine clearance, myocardial infarction without ST elevation. Angiographically type B and C lesions according to AHA/ACC, the uses of atherectomy devices, cutting balloon, hydrophilic guide wire, and oversize balloon and stent (balloon/artery size: 1,19+0,17) and post dilatation with high pressure emerge the risk of perforation.[1,3,7,13]

In coronary artery perforation, the most important factor designating mortality is the extensiveness of perforation. In grade III perforations, sudden onset tachycardia, chest pain, agitation, hypotension, increased jugular venous pressure, rapid development of tamponade may occur. In these patients, promptly inserted PTFE-covered stent prevents the development of sudden tamponade and cardiogenic shock.[10,14] Ellis et al. reported an incidence of tamponade of 63% and a mortality rate of 19% in coronary artery perforations.[3] In our study, in grade II and III perforations, the incidence of tamponade was 41,1% and the mortality rate was 11,7%. Both patients died had grade III perforations. The incidences of grade I, II and III perforations was reported to be 16,7%, 54,2% and %29,1, respectively.[15] In our study, we found the incidences of grade I, II and III perforations as 23,5%, 47,1% and 29,4%, respectively. Grade I perforations occur in association with guide wire, have a slowly progressing clinical course and good prognoses. They may be neglected since there was no extravasation, and may lead to the picture of tamponade even after 24 hours.

Despite the conventional treatment options are practicable in grade I and II perforations, these modalities are unsatisfactory for grade III perforations. The stent grafts are life-saving for these cases. Briguori C. et al. demonstrated that tamponade, emergency cardiac surgery and total major adverse cardiac events were significantly infrequent among the patients treated with covered stent. The rates of tamponade, emergency bypass surgery and death were 8%, 18%, and 2%, respectively, among patients implanted covered stent; whereas, these rates were reported as 82%, 88% and 35%, respectively, among cases given conventional therapy. In that study, full control of bleeding was achieved in 91% of the cases.[16] Alexandra J. et al. reported full control of bleeding in 92,8% of the cases with stent grafts.[15] In our study, we ensured TIMI 3 flow and full control of bleeding in 77,7% of the patients with stent graft. Currently, the most widely used stent grafts are Symbiot Stent (Boston Scientific), Josent Graft Stent (Abbott) and Nuvasc Graft Stent (Cardiovasc). [17] In our study, PTFE-covered JoStent Gretfmaster stents were used.

Rigid nature of stent grafts, demands of extra backup and high pressure seem to be their disadvantages. As a result of that, their success rates are low in distal, tortious and calcific lesions. More-

over, it was reported that with these stents, subacute stent thrombosis and restenosis were more frequent in comparison with other stents.[8]

Coil embolization seems to be an option in distal lesions, small vessels, chronic total occlusions, and in cases with perforation not allowing surgery.[18]

If extravasation persists despite of interventional processes, surgical operation is needed to ligate the perforated vessel, to implant bypass graft for distal perfusion, and to drain tamponade. Among the patients undergoing surgery, the rates of mortality and morbidity are rising depending on the time elapsed.[19] In our study, the rate of surgical mortality was 2/5 (40%).

In conclusion, coronary artery perforations most often occur in LAD, type B lesions, and as a consequence of balloon dilatations with high pressure. In perforations, clinical course and mortality is associated with extensiveness of perforation and therapeutic modalities implemented. Among the patients undergoing surgery, the rates of mortality seem to be very high probably due to the time elapsed. Therefore, the patient, as far as possible, should be treated in the catheter laboratory implementing stent graft and/or balloon tamponade procedures. The data indicates that there is a need for a more proper technology despite of the current treatment options.

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Evaluation of respiratory muscles' strength in patients with neuro-muscular diseases

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Abstract

We report a novel parameter for evaluating the degree of respiratory muscle failure from forced expiration records of patients with neuro-muscular diseases (NMD). Twenty-one NMD patients and six healthy subjects participated in this pilot study. The degree of respiratory muscle failure was evaluated with the Wright Professional Peak Flow Meter, modified to measure the dynamics of expired airflow. Three readings of peak expiratory flow (V_{max}), were made and the highest reading was selected as the person's representative value. From the ratio of V_{max} to the time to rise of V_{max} from 10% to 90% (T_r) we calculated a new parameter (V_{max}/T_r) or k_{exp}, for evaluating muscle failure during forced expiration. In NMD patients, this parameter was considerably smaller and significantly different than in healthy subjects (k_{exp}NMD = 54.54 l/s^2 ± 26 versus k_{exp} healthy = 193.55 l/s^2 ± 21). The value of k_{exp} decreases with the degree of respiratory muscle failure and the extent of respiratory muscle failure is related to the type and progression of the NMD. Respiratory function was most severely affected in patients suffering from Duchenne muscular dystrophy, limb-girdle muscular dystrophy and spinal muscular atrophy type 2. Comparing the V_{max}, T_r and k_{exp} values of patients and healthy volunteers, we conclude that the parameter k_{exp}, is the most sensitive indicator of the degree of respiratory muscle failure, due to its large dynamic range. Therefore, the parameter k_{exp} enables a more objective assessment of the progression of the NMD and could contribute to improved patient treatment.

Key words: Neuro-muscular diseases, respiratory muscle failure, forced expiration.

Introduction

In most neuromuscular diseases (NMD) progressive respiratory muscle weakness leads to respiratory insufficiency with life threatening complications.[1-6] Due to a reduced physical activity of patients with NMD, a respiratory insufficiency can often develop insidiously; therefore regular evaluations with functional pulmonary tests are vital.[3]

To prevent the disease from progressing rapidly, the NMD patients must be immediately included into a rehabilitation programme to monitor the progress of the disease and to treat complications such as contractures, scoliosis or breathing problems. It is essential to have up-to-date health information on the NMD in order to plan optimal treatment. A precise and sensitive quantification of motor deficit may also facilitate a better understanding of the natural course of NMD.[7-9] There is, as yet, no way of greatly affecting the long-term course of any NMD; therefore, to improve the patient's quality of life, treatment of NMD is symptomatic.

The aim of this study was to develop a novel parameter, derived from forced expiration records that would enable a better evaluation of the degree of respiratory muscle failure than conventional respiratory parameters. This would enable a more effective treatment of NMD patients and improve their quality of life.

Methods

Subjects

Twenty-seven subjects, 10 females and 17 males, were included in the study (Table I). The patient group comprised twenty-one NMD patients (11 men and 10 women), ranging from 9 to 54 years of age (mean age 29.54 years). Six healthy male subjects, age ranging from 9 to 54 years of age (mean age 28.33 years) represented the

control group. Among the 21 patients, 8 suffered from muscular dystrophy (Duchenne Muscular Dystrophy (DMD; n = 3), Limb-Girdle Muscular Dystrophy (LGMD; n = 3) and facioscapulohumeral muscular dystrophy (FSHMD; n = 2)). Thirteen patients suffered from other NMDs, not grouped among muscular dystrophies (Intermediate Spinal Muscular Atrophy (SMA2; n = 4), Juvenile Spinal Muscular Atrophy (SMA3; n = 5), Hereditary Motor and Sensory Neuropathy (HMSN; n=2), and Metabolic Myopathy (MM; n = 2)).

Equipment

The degree of respiratory muscle failure was evaluated by measuring the maximum flow rate of repeated, forced expirations with the Wright Professional Peak Flow Meter (WPFM, Ferraris Medical ltd; UK). The WPFM consists of a pivoted vane whose rotation inside a drum is opposed by air drag and a resistance spring. A radial inlet, into which a removable mouthpiece can be fitted, leads directly to the vane. A forced expiration through the radial inlet causes the vane to rotate, allowing the breath to escape the drum through an exhaust slot. The vane comes to rest at a position established by a peak airflow and is held in place by a roller clutch, which can be released by pressing a button adjacent to the radial inlet. A pointer registers the vane's movement on a scale calibrated in Litres per minute (LPM) which is graduated in 5 LPM segments over the range of 60 - 100. The WPFM also has a built-in one-way valve that prevents inhalation through the device. Along with the use of inexpensive disposable cardboard mouthpieces this valve reduces risk of infection among multiple users of the device.

A WPFM was modified to measure accurately both high and low ranges of air flow, and was thus suitable for use by adults and by children. For this purpose, the axel of the pivoted vane was fitted with a circular and transparent foil calibrated by 360 radial black lines extending from its circumference towards its centre. Thus the time-lapse could be measured when the pivoted vane rotated by increments of one degree. A sensor was constructed by mounting two infrared light-emitting diodes on one side of the foil and two photo transistors that detected the transmitted infrared light on the other side. In this way, the degree of rotation was measured. During the vane's rotation the calibrated foil activates the LEDs and phototransistors through interruptions of the transmitted infrared beam. The specifications of the electronic part of the Peak Flow Meter were: voltage regulated output ranging from 0 to ± 2.048 V, factor of transformation described as 1 revolution of a rotor/s = 500 mV at the output (a programmable variable), calculation time is 2 ms and resolution is 1 mV (11 bits). This electronic equipment was powered by an external 5V power supply and the output voltage was fed to a custom designed differential amplifier and to a DigiPack 1200, high performance data acquisition system connected to a personal computer. A Digidata 1200 data acqui-

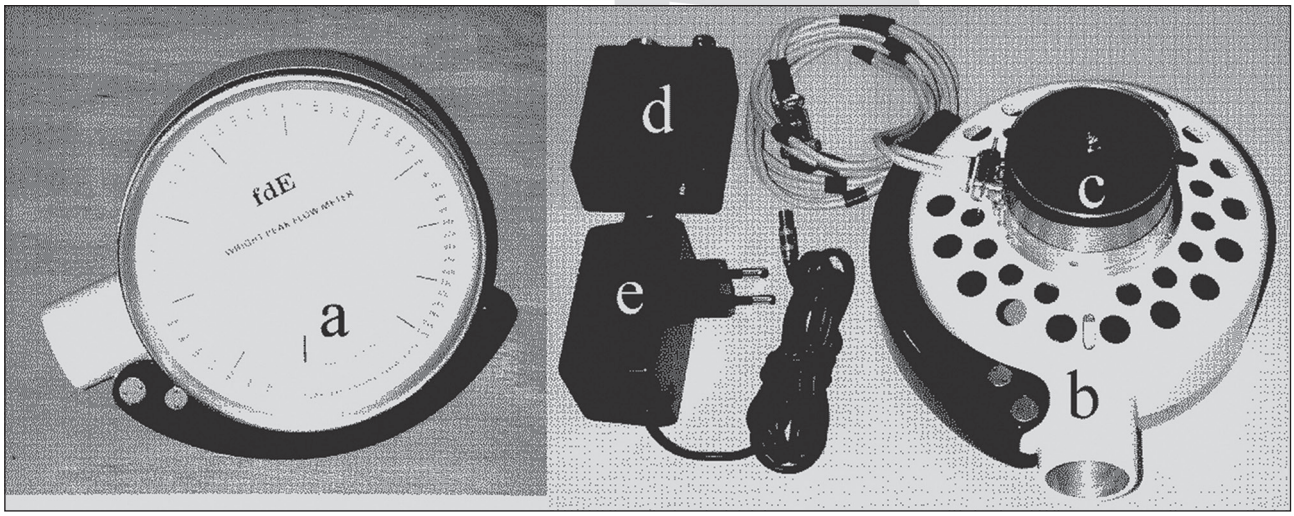

Figure 1. Wright Peak Flow Meter (FERRARIS MEDICAL LTD., a) front view, b) rear view, c) sensor, d) electronic part, e) power supply

sition system designed and manufactured by Axon Instruments and a high-speed data acquisition software were used for data analysis.

Peak Expiratory Flow (V_{max}) Measurement

A verbal, informed consent was obtained from all study participants before any test was performed. All the procedures adhered to the standards of the Committee on Human Experimentation of the University Rehabilitation Institute, Republic of Slovenia, which are in accordance with the Helsinki Declaration of 1975.

The measurement was performed with the test subject sitting upright in a chair or a wheelchair. The WPFM was held as presented in Figure 2.

Figure 2. The Peak Flow Meter held by a patient sitting upright

The WPFM was held in a vertical plane with the dial facing right and the exhaust vent obstructed (Figure 2). It was essential to give test subjects clear instructions about the procedure. The test administrator first demonstrated the test by performing it himself. As in all simple tests of respiratory capacity, it was important to persuade patients to make a maximal effort. During measurements patients were provided with a visual display of peak-air-flow and also orally encouraged to make a maximum effort.

After maximal inspiration, the test subject gripped the mouthpiece lightly with the teeth and sealed it with the lips. He/she then made a forced expiration, as quickly as possible, in a short, sharp blast, using all the respiratory muscles. The person was monitored to ensure that no leaks occurred between the mouthpiece and the lips. Since most of our test subjects were new to this procedure, the measurements were preceded by a practice trial that was repeated until the test was performed correctly. Once the test was correctly performed, three readings of V_{max} were made, each lasting about 300 ms. For the sake of convenience and following current practice, the test was administered as a series of 3 trials for each person. The results of three trials were recorded and the highest reading was used in analysis of the subject's V_{max}. If the test subject had a moderate or severe respiratory insufficiency an adequate period of rest between attempts was allowed. Measurements were separated by 30 s to 60 s long intervals. In order to minimize any reduction in V_{max} due to muscle fatigue the interval was varied between subjects, and in some cases an interval of at least a minute was allowed for recovery.

Analysis of Peak Expiratory Flow measurements

An example of a V_{max} record of a SMA-3 patient is shown in Figure 3.

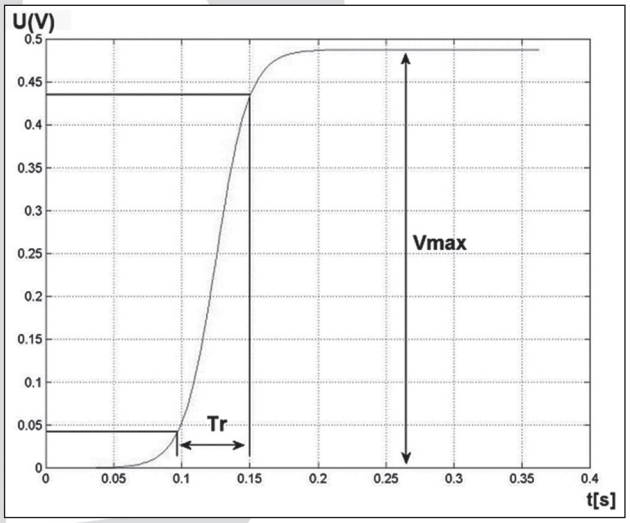

Figure 3. A time course of forced expiration to V_{max} in a patient with SMA-3. Abbreviations: V_{max} (peak expiratory flow during forced expiration units in mV); T_r (rise-time from 10% to 90% of V_{max}).

We introduced a new parameter, parameter *k*, representing a ratio between V_{max} and T_r, as shown in equation (1):

$$k = \frac{v_{max}[mV]}{T_r[ms]} \quad \text{..... (1)}$$

The value of V_{max} (units in V) was calibrated and expressed in the more conventional units of litres per minute (l/min) by multiplying the V_{max} value expressed in V with the transformation factor 0.91 (units in l/V min) which represents a factor of transformation between mechanical and electronic part of the WPFM. Accordingly, in equation (2), *k* was recalculated:

$$\frac{v_{max}[l/min]}{T_r[ms]} = \frac{v_{max}[l]}{T_r[60s \cdot 0.001s]} = \frac{v_{max}}{0.06T_r}[l/s^2] \quad \text{..... (2)}$$

Since the unit [l/s^2] represents the volumetric acceleration one could conclude that the parameter *k* is a good measure of the degree of muscular weakness and thus reflects the functional state of respiratory muscles in patients with NMD. However, the parameter *k* represents an approximation of the average volumetric acceleration of expired air during forced expiration.

Results

The results are summarized in Tables 1 and 2. Table 1 gives the values for V_{max}, T_r and k_{exp} for each healthy volunteer and for the 21 patients

Table 1. Forced expiration parameters of patients with neuro-muscular diseases: DMD (Duchenne muscular dystrophy), FSHMD (facioscapulohumeral muscular dystrophy), LGMD (limb-girdle muscular dystrophy), MM (metabolic myopathy), SMA2 (spinal muscular atrophy type 2), SMA3 (spinal muscular atrophy type 3), HMSN (hereditary motor and sensory neuropathy). Abbreviations: Vmax (peak expiratory flow during forced expiration); Tr (rise-time from 10% to 90% of Vmax); kexp (new parameter for evaluating respiratory muscle strength).

Subject	Gender	Diagnosis	Age	V_{max} [l/s]	T_r (s)	k_{exp} [l/s^2]
4	M	DMD	9	1.63	0.09	17.89
15	M	DMD	21	2.77	0.08	33.09
20	M	DMD	13	2.69	0.10	28.24
2	M	FSHMD	21	4.67	0.07	66.19
17	F	FSHMD	28	4.97	0.08	60.57
9	F	HMSN	21	4.06	0.09	45.49
19	M	HMSN	32	5.99	0.07	82.91
7	F	LGMD	31	2.47	0.11	22.25
18	M	LGMD	48	4.21	0.10	41.17
21	M	LGMD	26	5.84	0.10	60.86
10	M	MM	30	6.60	0.11	59.47
12	F	MM	23	7.25	0.09	78.17
1	F	SMA2	27	3.15	0.09	36.01
3	F	SMA2	17	3.15	0.08	41.49
5	F	SMA2	54	5.08	0.07	68.20
11	F	SMA2	32	4.06	0.11	37.38
6	M	SMA3	35	6.41	0.07	88.69
8	F	SMA3	34	3.79	0.08	45.33
13	M	SMA3	38	7.44	0.07	106.39
14	M	SMA3	52	3.87	0.17	22.79
16	F	SMA3	28	7.85	0.08	102.86
22	M	HEALTHY	26	8.50	0.04	193.15
23	M	HEALTHY	25	10.17	0.05	195.54
24	M	HEALTHY	26	10.30	0.05	190.83
25	M	HEALTHY	26	11.08	0.05	213.05
26	M	HEALTHY	21	10.59	0.05	213.20
27	M	HEALTHY	46	9.33	0.06	155.56

with NMD. Compared to healthy subjects, all of the NMD patients have smaller V_{max} and k_{exp} values and larger T_r values (Table 1). These observed differences were statistically significant ($P < 0.001$ two-tailed, unpaired Student test t, 99% confidence interval; top section of Table 2). Although the average V_{max}, T_r and k_{exp} values are significantly different between the healthy volunteers and the patients group they do not have the same dynamic range (DR) − the ratio between the largest and smallest values. The k_{exp} has the largest dynamic range, and the T_r the smallest. This difference is more pronounced in the patients group than in the healthy volunteers group (V_{max} DR = 4.81; T_r DR = 1.94; k_{exp}DR= 5.95; bottom section of Table 2). The largest dynamic range of parameter K_{max} is consistent with the largest ratio between the average values of healthy volunteers and NMD patients. The ratio between average values of patients and healthy volunteers is 3.5 for K_{exp}, 2.14 for V_{max} and 1.8 for T_r (the largest value is always the dividend).

Discussion

This aim of our study was to introduce a novel parameter for monitoring the level of respiratory muscle failure during forced expiration in patients with NMD. Apart from measurements of V_{max} with a conventional WPFM during forced expiration, we also recorded the time build-up, or dynamics of V_{max} with a modified WPFM. We measured the rise time between 10% and 90% of peak expiratory flow (Tr) in healthy subjects and in patients with NMD. The recorded Tr values suggest it's a good indicator of the level of muscular weakness. We also introduced the parameter k_{exp}, which compared to V_{max} and T_r has the largest, significant difference between patients with NMD and healthy people (k_{exp} = 54.54 ± 26.1 and 193.55 ± 21 respectively). Compared to V_{max} and T_r, the parameter k_{exp} also has the largest dynamic range, thus enabling a more accurate measurement of the NMD's progress.

Since most NMD affect both inspiratory and expiratory muscles, patients with NMD should be referred to pulmonary specialists before they develop severe respiratory impairment. The clinician has access to a number of tools for assessing the respiratory function of NMD patients. Attention to the detailed history and the presence of certain symptoms and signs can provide the first clues that respiratory muscle weakness is present. This can be followed by a global respiratory muscle assessment using measurements of total lung capacity (TLC),

Table 2. The average values ± SD of Vmax, Tr and Kexp, for patients and healthy volunteers, are presented in the top section. The differences between healthy volunteers and patients are statistically significant for all the parameters (P < 0.001; two tailed and unpaired Student test t). The bottom section gives the values for the dynamic ranges (DR) of these parameters. Compared to V_{max} and T_r, K_{exp} has the largest DR for pooled data of patients with different NMD and for healthy volunteers (data shaded in gray). The only exception are the data of patients with FSHMD, where T_r has the largest DR. Legend to patients' abbreviations is the same as in Table 1.

	n	V_{max} [l/s]	T_r (s)	k_{exp} [l/s²]
HEALTHY (H)	6	9.99 ± 0.93	0.05 ± 0.01	193.55 ± 21.1
PATIENTS (P)	21	4.66 ± 1.78	0.09 ± 0.01	54.54 ± 26.1
H : P	6 : 21	P < 0.0001	P < 0.0001	P < 0.0001
	n	V_{max} DR	T_r DR	k_{exp} DR
(H)	6	1.36	1.30	1.37
(P)	21	4.81	1.94	5.95
DMD	3	1.70	1.14	1.85
FSHMD	2	1.07	1.16	1.09
HMSN	2	1.48	1.23	1.82
LGMD	3	2.37	1.15	2.74
MM	2	1.10	1.20	1.31
SMA2	4	1.61	1.46	1.89
SMA3	5	2.07	2.43	4.67

vital capacity (VC), maximal static inspiratory and expiratory pressures (PI_{max} and PE_{max}), and nasal sniff pressures. These tests are often non-specific but are readily available and provide further estimates of respiratory muscle impairment.[10-12] Specific tests of diaphragm function such as transdiaphragmatic manometry can be utilized but may be limited because they are invasive and uncomfortable.[13] Finally, newer techniques such as electrical and magnetic stimulation of the phrenic nerves or ultrasound of the diaphragm in the zone of apposition may become available as their utility is demonstrated in various types of NMD.[13] Blood gas analysis is an important part of respiratory evaluation in NMD patients. Hypoxemia occurs in advanced disease and is usually multifactorial. First, alveolar hypoventilation increases pCO_2 and reduces pO_2.[12, 14, 15-21] There is no single approach that suits all NMD patients with respiratory dysfunction. The course and pattern of respiratory dysfunction differ markedly between the various types of NMD and even between patients with the same NMD. Therefore, therapy must be individualized.

The quantification of respiratory insufficiency in patients with NMD has recently become more interesting to investigators; however the techniques for evaluating the progress of respiratory insufficiency have to be further developed. Therefore optimal treatment of NMD cannot be achieved, since the physician cannot closely follow the effect of treatment on the progress of the disease. Assessment of muscle strength is the most direct measure of motor deficiency and manual muscle testing is the most frequently used method for assessing muscle strength of certain skeletal muscles or muscle groups.[22, 23] However, there is no direct method to measure the force of respiratory muscles' contraction. The functional state of expiratory muscles can be estimated from the volume of air exhaled during the first second of forced exhalation (FEV1). In a healthy subject, FEV1 depends mainly on the functional state of the expiratory muscles and the diameter of the upper respiratory apparatus. In patients with NMD, FEV1 relates directly to maximal respiratory effort. Accordingly, forced expiratory flow rates are reduced in patients with NMD.[24, 25] The functional state of expiratory muscles can be assessed by using a Peak Flow Meter (PFM), which measures the Peak Expiratory Flow (PEF designated as V_{max} in our results) during a forced expiration. The PEF is a non-invasive test of expiratory muscle strength and is easy to perform on patients with NMD.[24] PEF is defined as the maximum flow rate maintained for at least 10 ms during a forced expiration. For our study we used the analogue PFM since the digital models cannot be modified to give a T_r reading.

Conclusion

We conclude that, compared to V_{max} and T_r, parameter k_{exp} enables a more objective assessment of respiratory muscle weakness in patients with NMD due to its large dynamic range. Therefore the parameter K_{exp} could be considered an additional, useful parameter in rehabilitation programmes provided its properties are validated on a larger sample of patients and healthy subjects.

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The role of allergic rhinitis in the development of otitis media with effusion

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Abstract

Objective: Otitis media with effusion (OME) is a common disease in children with an incidence of 15% to 20% and which may lead to hearing loss or surgical intervention. In most children, otitis media improves spontaneously and has a good prognosis, but in addition to hearing loss, chronic OME may be associated with learning difficulties and delay of language development. Although numerous studies have been performed to determine the relationship between allergy and OME, the incidence of AR in OME has been found to vary from 14% to as high as 89%, making it the role of allergic rhinitis in middle-ear disease is not fully understood. The association between AR and OME has not yet been evaluated in Turkey. We therefore prospectively determined the association between AR and OME and the effect of AR on eustachian tube function in pediatric patients.

Methods: The subject group consisted of 62 pediatric patients who diagnosed as allergic rhinitis (AR group), and 67 healthy children applied to the hospital. Every child was evaluated with detailed medical history and physical examinations were performed, including anterior rhinoscopy, otoscopy, skin prick test, hemogram, serum IgE level measurement and impedance audiometry.

The tympanographic trace was classified according to basic curve types described by Liden and Jerger: Type A, normal eustachian tube function; Type B, flat tympanogram characteristic of the presence of fluid in the middle ear, eardrum perforations, or impacted cerumen; Type C, characterized by peaks at very negative pressures, typically -150 daPa, and may be indicative of eardrum retraction.

Results: Of the 62 patients in the AR group, 40 (64.5%) were diagnosed with eustachian tube dysfunction(type B or C), whereas 30 (44.8%) of 67 patients in the control group were diagnosed with eustachian tube dysfunction; the difference was significant (p=0.034). By Type B tracing and physical examination, SOM were diagnosed 27(43.5%) of the 62 patients in the AR group, whereas 26(38.8%) of 67 patients in the control group were diagnosed with type B tracing and physical examination; the difference was not significant (p=0.596). By Type C tracing and physical examination, Eardrum retraction were diagnosed 22(35.5%) of the 62 patients in the AR group, whereas 6(9%) of 67 patients in the control group were diagnosed with type C tracing and physical examination; the difference was significant (p<0.001). The average eosinophil count was 8.3±2.8% in the AR group and 6.2±0.6% in the control group (P=0.504; Fig. 1); the average serum IgE concentration was 497±72mg/dlt in the AR group and 233±77 mg/dlt in the control group (P=0.023).

Conclusion: Otitis media with effusion which may lead to hearing loss or surgical intervention is one of the most common infection for which antibiotics are prescribed for children and contribute a great deal and health care costs. However, the studies on the theoretical mechanism, the relation between allergy and otitis media with effusion is stil controversial. Althought we have found no difference in incidence of OME and blood eosinophil counts in pediatric patients with and without AR, chronic complications of OME and concentration of serum IgE is found significantly higher in incidence of OME with AR. We beleive that our results suggesting that AR indirectly leads to chronic OME by inhibiting resolution of OME rather than causing directly the developement of OME. Before saying antiallergy therapy is efficacious in preventing or limiting the duration of otitis media with effusion that we should screen every child with otitis media with effusion for allergic rhinitis, well controlled clinical studies should be conducted documenting that in selected populations.

Key words: Allergic Rhinitis, Serous Otitis Media, Tympanogram.

Introduction

Otitis media with effusion (OME) is a common disease in children with an incidence of 15% to

20% and which may lead to hearing loss or surgical intervention [1]. Approximately 90% of children (80% of individual ears) have OME at some time before school age [2]. In most children, otitis media improves spontaneously and has a good prognosis, but in addition to hearing loss, chronic OME may be associated with learning difficulties and delay of language development [3,4]. Among the major causes of OME are upper respiratory tract infection and dysfunction of the eustachian tube. Otitis media with effusion may also be caused by a combination of the host immune system, environmental factors, family history, and allergies, among other factors.

A number of mechanisms have been proposed for the development of allergen-induced OM, including the middle ear functioning as an allergic "shock organ," inflammatory swelling of the eustachian tube, and inflammatory obstruction of the nose and secondary eustachian tube dysfunction [5].

Although numerous studies have been performed to determine the relationship between allergy and OME, the incidence of AR in OME has been found to vary from 14% to as high as 89%, making it the role of allergic rhinitis in middle-ear disease is not fully understood [6,7,8].

The association between AR and OME has not yet been evaluated in Turkey. We therefore prospectively determined the association between AR and OME and the effect of AR on eustachian tube function in pediatric patients.

Methods

The subject group consisted of 62 pediatric patients (32 boys, 30 girls), ranging in age from 5 years to 12 years (mean ± SD age, 7.5 ± 2.3 years), who diagnosed as allergic rhinitis (AR group), and 67 control children (41 boys, 26 girls), ranging in age from 5 years to 15 years (mean ± SD age, 8.2 ± 2.7 years), treated in the Department of Pediatric Allergy and the Department of Ear, Nose and Throat from April 2007 to november 2007.

Materials and methods

Subjects

The subject group consisted of 62 pediatric patients (32 boys, 30 girls), ranging in age from 5 years to 12 years (mean ± SD age, 7.5 ± 2.3 years), who diagnosed as allergic rhinitis (AR group), and 67 control children (41 boys, 26 girls), ranging in age from 5 years to 15 years (mean ± SD age, 8.2 ± 2.7 years), treated in the Department of Pediatric Allergy and the Department of Ear, Nose and Throat from April 2007 to november 2007. The AR group consisted of children with no history of OME or ear symptoms but who had symptoms such as sneezing, watery rhinorrhea, nasal obstruction, and pale or watery nasal mucosae as detected by physical examination, and a positive reaction to house dust, Dermatophagoides farinae, or cockroach mix in allergy skin reaction tests. Children diagnosed with AR had received no medications during the previous 2 weeks and had no history of immunotherapy. The control group were healthy subjects, selected among children of outpatients of the same institution whose parents agreed to participate in the study. Children with adenoid, congenital anomalies in the head and neck, Down syndrome, systemic diseases, or those suspected of having congenital or acquired immune deficiency were excluded from this study. The study protocol was fully explained to patients or their guardians, and written informed consent was obtained from each patient.

Study design

At the time of first visit by each patient, a detailed medical history was obtained, and physical examinations were performed, including anterior rhinoscopy, otoscopy, impedance audiometry. the AR group was diagnosed by taking a detailed medical history, performing anterior rhinoscopy, and allergy skin reaction as well as tympanometry. Blood samples obtained from patients were used, IgE levels were measured with *Immunochemistry Systems* (Beckman Coulter, U.S.A that uses the principle of nephelometry, and for eosinophil count, hemograms were analysed with a Sysmex XT 2000i (Sysmex Corporation, Kobe, Japan). Otitis media with effusion was diagnosed by the presence of amber color, air-fluid, or air bubbles in the middle ear as determined by otoscopy, and by tympanometry. Tympanometric peak pressure (TPP) that is the peak admittance in the tympanogram and constitutes an indirect measurement of the middle ear pressure through the intact eardrum. Figures under 100 daPa are considered as negative curves that reflect an impaired eustachian tube function.

The tympanographic trace was classified according to basic curve types described by Liden [9] and Jerger[10]: Type A, normal eustachian tube function; Type B, flat tympanogram characteristic of the presence of fluid in the middle ear, eardrum perforations, or impacted cerumen; Type C, characterized by peaks at very negative pressures, typically -150 daPa, and may be indicative of eardrum retraction. Parameters obtained with pediatric table based on Palmú's[11] criteria were adjusted in children under 11 years old. The cases and controls were stratified by age groups.AR was diagnosed by watery rhinorrhea, nasal obstruction, sneezing, nasal pruritus, and 2+ more positivity in the MAST-CLA and/or allergic skin test.

Analysis was performed using SPSS 17.0 version for windows software with descriptive statistics and tests for hypotheses to compare 2 independent samples.

Allergic skin test

Skin reaction tests were performed on the forearm or back of each patient using prick test reagents. Solutions of histamine and saline were used as positive and negative controls, respectively. The longest diameter and the diameter perpendicular to it were measured for each wheal and erythema response, with the averages considered the response size, and positivity was determined by the ratio of each to that of the positive control. Wider reactions comparable to the size and enduration of the histamine control solution were determined to be positive.

Results

Of the 62 patients in the AR group, 40 (64.5%) were diagnosed with eustachian tube dysfunction(type B or C), whereas 30 (44.8%) of 67 patients in the control group were diagnosed with eustachian tube dysfunction; the difference was significant (p=0.034); Figure 1. By Type B tracing and physical examination, SOM were diagnosed 27(43.5%) of the 62 patients in the AR group, whereas 26(38.8%) of 67 patients in the control group were diagnosed with type B tracing and physical examination; the difference was not significant (p=0.596); Figure 2. By Type C tracing and physical examination, Eardrum retraction were diagnosed 22(35.5%) of the 62 patients in the AR group, whereas 6(9%) of 67 patients in the control group were diagnosed with type C tracing and physical examination; the difference was significant (p<0.001); Figure 3. The average eosinophil count was 8.3±2.8% in the AR group and 6.2±0.6% in the control group (P=0.504; Figure 4); the average serum IgE concentration was 497±72mg/dlt in the AR group and 233±77 mg/dlt in the control group (P=0.023; Figure 5).

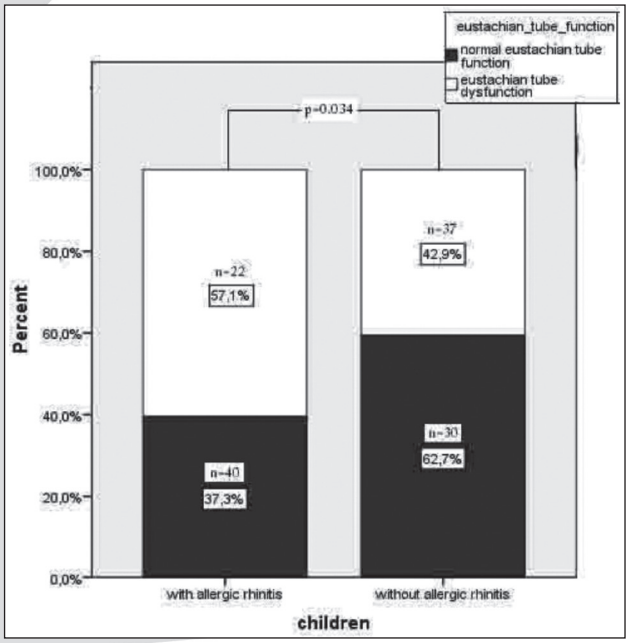

Figure 1. Prevelance of abnormal eustachian tube function, as determined by tympanometry, in the AR and control groups. Significant difference was found (p=0.034)

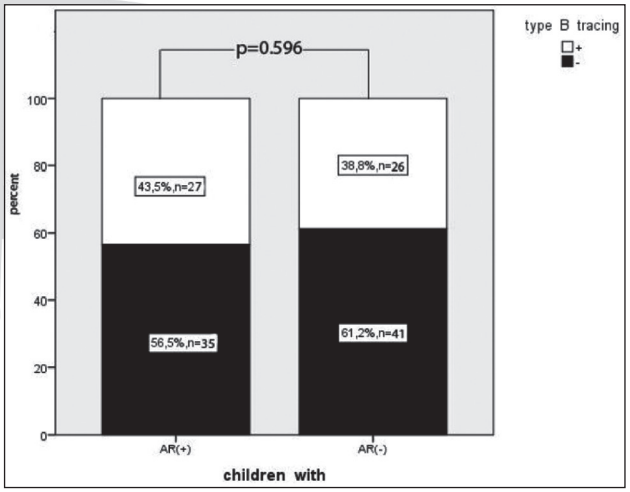

Figure 2. Prevelance of abnormal Type B tracing, as determined by tympanometry, in the AR and control groups. Significant difference was not found (p=0.596)

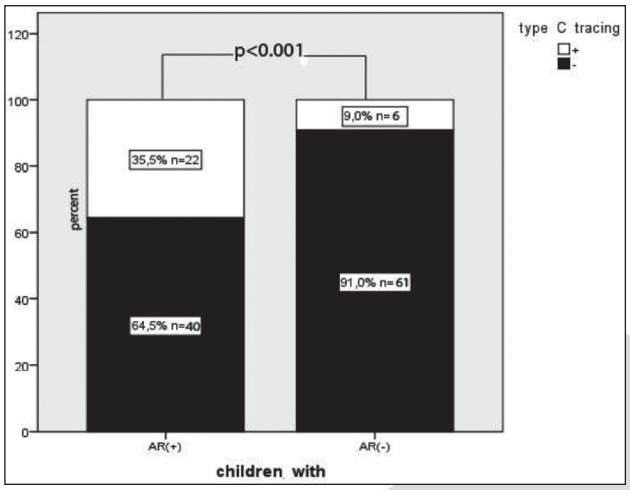

Figure 3. Prevelance of abnormal Type C tracing, as determined by tympanometry, in the AR and control groups. Significant difference was not found (p<0.001)

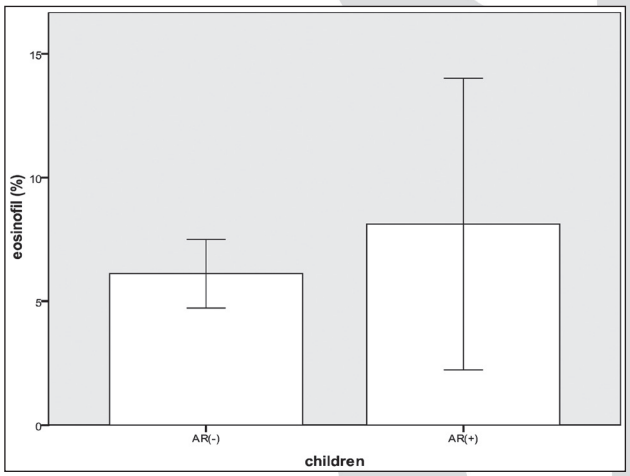

Figure 4. Eosinophil counts in the AR and control groups. No significant difference was observed (P>0.05)

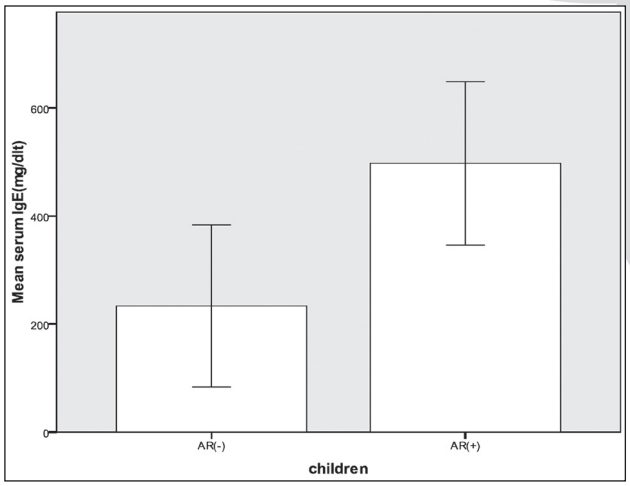

Figure 5. Serum IgE concentrations in the AR and control groups. Significant difference was found (P=0.023)

Discussion

The incidence of OME in children with AR has been reported to range from 14% [12] to 89% [13]; however, in both of these studies, the diagnosis standards were vague, and there were no control groups. One study comparing the incidence of AR in OME and control groups found a significant difference [14], whereas a second did not [15].In these studies, however, the standard for diagnosis of allergy was vague, they did not use the formally established definition of AR, and the incidence of AR in patients with OME was dependent on the intention of the study, suggesting flaws in their design [16-18].

Althought sample size is small in number, we beleive that our study give the more reliable estimates when compared the those similar sudies; because of our study is prospective, and we have adopted rigorous diagnostic criteria, again in our study adequate random control group population is existed and because of congenital anomalies and children younger than 5 years of age which may be associated with the shorter length and flatter position of the eustachian tube relative to the posterior nasopharyngeal wall compared with that of older children were excluded.

Our results with clinical examination, show that eustachian tube function abnormalities among a group of allergic rhinitis patients were greater than that of healthy subjects (p<0.05). But when we evaluate those abnormalities seperately as type B and type C; results have gained different meanings.

In our study, Type B, flat tympanogram characteristic of the presence of fluid in the middle ear which was also confirmed by physical examination is found in similar ratios in both AR and control groups(p>0.05). When we accept the presence of Type B Flat tympanogram as the indicative sign of OME existence, we may suggest that an absence of direct association between AR and OME. It is controversery to many studies, it is suggested that allergic diseases of the upper respiratory tract are becoming one of the leading factors in the etiology of Eustachian tube dysfunctions and this dysfunction is the most significant etiologic factor of secretory otitis media.

Intranasal relevant allergen challenge performed on human adults with allergic rhinitis and on sensitized monkeys, rats caused eustachian tube

dysfunction. Challenge tests on patients without allergies or rhinitis with allergens did not provoke these responses [19, 20, 21, 22, 23]. These signs and symptoms were reproduced by means of intranasal challenges with mediators of inflammation esppecially by histamine released or synthesized during an allergic reaction [24, 25, 26,27].

But, none of these studies resulted in sustained middle ear underpressures which is necessary for development of OM. Additional studies have documented eustachian tube dysfunction and middle ear underpressures in patients with allergic rhinitis during natural allergen exposure, but the development of OM was rare [28,29]. Recently Yeo and his friends has suggested that allergic rhinitis may not be related to the developement of OME in children, similarly to our results [39].

It is clear that upper respiratory track allergy may cause some intrinsic and extrinsic mechanical obstruction but posibbly active opening mechanism (TVP muscle pull) overcomes the obstruction. And this prevents sustained eustacian tube dysfunction and developement of OM.

Type C, characterized by peaks at very negative pressures, typically -150 daPa, and may be indicative of eardrum retraction is found higher in AR group than healthy group($p<0.05$)). When we accept the presence of Type C Flat tympanogram as the indicative sign of chronic OME existence and apperance of complications, also confirmed by physical examination we may suggest that the presence of direct association between AR and chronic OME. Our results do not support such general idea that allergic rhinitis is one of the major precursor in the developement of OME. Rather than leading cause, Our data supports that AR inhibits resolution of the OME and lead to the chronic complications of the OME. Relationship between potential viral pathogenic mechanisms and AR may explain this suggestion.

Viral URIs are the most common infections among human beings and occur between two and four times per year among adults and much more frequently among children [43]. For OM, approximately 50% of new episodes are diagnosed immediately after or during a viral URI[44, 45, 46].

With middle ear effusion bacterial recovery rates of 70% for acute OM and 30% to 50% for persistent OM.[47,48] and as with viruses, these rates increase when a diagnostic polymerase chain reaction assay is used [49]. In several studies [47, 50], approximately 50% of chronic,persistent middle ear effusions had positive cultures for bacteria with microbiologic features similar to those in acute OM.

Viruses that cause URIs alter respiratory epithelial receptors and have a differential effect on bacterial adherence and the bacterial flora of the nasopharynx. Presence of AR may predispose the patient to develop more frequent and severe URTI by inducing the expression of adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) on epithelial cells [33]. Upregulation of the expression of ICAM-1, the principal receptor for rhinovirus, might increase tissue susceptibility to infection with rhinovirus. In addition, epithelial cells from asthmatic patients show a deficient innate immune system, hence favouring viral replication and invasion [34]. It is also known that atopic children with asthma experienced more common colds than non-atopic asthmatic children, without difference in severity or duration of the common colds [35]. Computerized tomography [CT] studies reveal that patients with AR have more severe paranasal sinus changes during viral colds than nonallergic individuals [36, 37], concomitant with a reduced mucociliary clearance time. In the light of this observation, IL-13, which is a key cytokine in allergic airway inflammation, reduces ciliary beat frequency [38] by slowing down mucociliary clearance, thereby favouring viral invasion of the mucosa.

These changes may promote the development of a secondary bacterial infection of the middle ear. These observations and epidemiologic data that indicate many episodes of OM are preceded by or associated with a clinical illness typical of a viral URI have led investigators to suggest that viral URIs interact with bacterial infections in promoting OM. Viral effects that can promote bacterial infection include altered bacterial adherence [51,52,53] modulation of the host immune and inflammatory response [54,55,56] and impaired eustachian tube function.64, 65 In different studies it is demonstrated that IgE-mediated reaction may be persisting factor of OME due to disturbance of the clearance of MEE by the eustachian tube [41,42].

MEE resolve spontaneously within 3 months, but 30% to 40% of children have recurrent OME, and 5% to 10% of episodes last 1 year or longer

[30,31,32]. Because of an inadequate host defense can contribute to recurrent respiratory infections and chronic distruption of the eustachian tube function, AR may be a possible distruptive factor in spontaneous resolution phase.This may explain why allergic patients tend to develope more chronic complications of OME while no difference is found in OME prevelance between allergic and healthy children.

IgE antibody constitutes the first line of defense against microorganisms. IgE antibody specific for bacterial or viral antigens is thought to act by localizing high concentrations of protective antibody at the site of tissue invasion [57]. Thus, serum total IgE concentration in adults and children with AR and asthma is high. But it has a low sensitivity in the differentiation between atopy and non-atopy in young children [58]. According to these authors, high levels suggest atopy, while normal or low values yield little information. In animals, stimulation of the middle ear with pollen antigens has been found to induce inflammation in the middle ear, suggesting that the middle ear mucosa may be effected by type I reaction and IgE could be produced locally by the middle ear mucosa if it was the target organ[59].

We found that the concentration of serum IgE children with AR is significantly higher than children without AR which may be also indicative sign of local mucosa IgE production like nasal mucosa, sinuses, milddle ear mucosa[60,61,62,63], suggesting that middle ear cavity may be affected by local tissue Type I allergic reactions. The eosinophil comprises approximately 2% to 5% of granulocytes in a person without allergies. There has been increasing awareness of the role of the eosinophil in several physiologic and pathologic processes. Activated eosinophils have been found to play a role in allergy, asthma, parasitic diseases, granulomatous disorders, fibrotic conditions, and several malignant tumors.

We found no significant difference in blood eosinophil count between the AR and control groups of children. Both groups blood eosinophil counts are higher than 5% and both groups may be accepted as having high eosinophil counts. Although an increase in eosinophils is common in bronchial asthma and atopic dermatitis, in our country another common high eosinophil count reason is helminthic infections[40]**.** This may be causative reason in such high eosinophilic counts in both groups and it makes interpretation of this parameter is harder.

Otitis media with effusion which may lead to hearing loss or surgical intervention is one of the most common infection for which antibiotics are prescribed for children and contribute a great deal and health care costs. However, the studies on the theoretical mechanism, the relation between allergy and otitis media with effusion is stil controversial. Althought we have found no difference in incidence of OME and blood eosinophil counts in pediatric patients with and without AR, chronic complications of OME and concentration of serum IgE is found significantly higher in incidence of OME with AR. We beleive that our results suggesting that AR indirectly leads to chronic OME by inhibiting resolution of OME rather than causing directly the developement of OME.

Before saying that we should screen every child with otitis media with effusion for allergic rhinitis, well controlled clinical studies should be conducted documenting that in selected populations antiallergy therapy is efficacious in preventing or limiting the duration of otitis media with effusion.

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Endometrial carcinoma and association of high-mobility group box 1 expression levels in cytoplasm

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Abstract

Background: To investigate the expression pattern of high-mobility group box 1 (HMGB1) in endometrial carcinoma and hysteromyoma.

Methods: This study was performed at the Department of Obstetrics and Gynecology, XiangYa Hospital, Central South University, Changsha, China. One hundred sixteen patients with adenocarcinoma or hysteromyoma and 38 subjects with infertility donated endometrium tissue. Six types of tissue, namely proliferative phase endometrium, secretory phase endometrium, well-differentiated endometrium, poorly-differentiated endometrium, hysteromyoma, and normal myometrium were evaluated. HMGB1 expression and protein distribution were detected by real-time polymerase chain reaction and immunohistochemistry, respectively, and compared among the three groups of subjects regarding their percentage and intensity of immunostaining.

Results: The expression of HMGB1 protein is negative or weakly positive in the normal and proliferative phase endometrium. In endometrial carcinoma, expression level of HMGB1 is significantly increased compared with normal tissue. Interestingly, expression of HMGB1 is highest in the cytoplasm of endometrial carcinoma, while expression of HMGB1 is highest in the nucleus of hysteromyoma.

Conclusion: HMGB1 is highly expressed in endometrial carcinoma, and cytoplasmic HMGB1 may contribute to the development of endometrial carcinoma. Monitoring expression of HMGB1 in endometrial tissues may help diagnosis or prognosis of endometrial carcinoma.

Key words: Endometrial Carcinoma, HMGB1, Immunohistochemistry, RT-PCR, Western blot.

Introduction

High-mobility group box 1 (HMGB1) was originally identified as a highly conserved nuclear protein, acting as a chromatin-binding factor, to promote physical interactions between DNA and its binding factors, including p53, NF-κB and steroid hormone receptors (1,2). Over-expression of HMGB1 is associated with each of the features of cancer including unlimited replicative potential, insensitivity to inhibitors of growth, ability to develop blood vessels, evasion of programmed cell death, self-sufficiency in growth signals, inflammation, and tissue invasion and metastasis (2). Previous studies have shown that over-expression of HMGB1 strongly correlates with tumor growth and invasiveness in different cancers(3-7). However, in the case of chemotherapy or radiotherapy, extracellular HMGB1 can induce anti-tumor immunity in a tumor microenvironment (8).

Endometrial adenocarcinoma is the seventh most common cancer among women. Its incidence in developed countries is ten times higher than in developing countries (9,10). The mechanisms involved in the development of endometrial cancer remain unknown although some factors, such as hormonal over-stimulation, are suspected (11,12).

As the level of HMGB1 is elevated in breast, colorectal, prostate, lung and hepatocellular carcinoma (3-7), we hypothesized that the level of HMGB1 is also increased in endometrial adenocarcinoma. Therefore, in this study, we compared the levels of HMGB1 in the tissues of endometrial adenocarcinoma and hysteromyoma, as well as normal endometrial tissues.

Methods

Patients and controls

The adenocarcinoma and hysteromyoma tissues were obtained from 116 patients in the Department of Gynecology of Xiangya Hospital, Hunan, China. Normal endometrial tissue was collected from 38 subjects with infertility, including 20 people with proliferative phase endometrium, and 18 in the secretory phase. The study was

approved by the ethical committee of the hospital and written informed consent was obtained from each participant and conducted in accordance with The Code of Ethics of the Declaration of Helsinki.

RNA extraction and real-time polymerase chain reaction analysis

Total RNA was isolated with TRIzol reagent (Invitrogen, Shanghai, China). cDNA was prepared by Oligo(dT)$_{12-18}$ and reverse transcriptase SuperScript II from Invitrogen (Shanghai, China) with 2μg of DNase I-treated total RNA. For real-time polymerase chain reaction, 2μl of the cDNAs was added to an 18μl polymerase chain reaction assay mixture containing 0.5M each primer and 10μl Brilliant SYBR® Green QPCR Master Mix (Stratagene, California, USA). Polymerase chain reaction was conducted with the MyiQ Single Color Real-Time Polymerase Chain Reaction Detection System (Bio-Rad, Shanghai, China) using the following conditions: the cycle was started at 95°C and lasted for 10 min, followed by 40 cycles of 95°C for 15s, 55°C for 30s, and 72°C for 30s. Two sets of polymerase chain reaction assays were performed for each sample using the primers for HMGB1 (forward primer 5'-GTAGAATTCATGGGCAAAGGAGATCCTA-3' and reverse primer 5'-AGGCTCGAGTTCATCATCATCATCTTCT-3'), and β-actin (forward primer 5'-TAACTGGAACGGTGAAGGTG-3'). The threshold cycle number for HMGB1 was normalized to that of β-actin, and the resulting value was converted to a linear scale. All assays were performed at least three times from independent RNA preparations.

Western blot analysis

For protein analysis, samples were homogenized in lysis buffer (150mM NaCl, 50mM Tris-Cl, pH 8.0), 5mM EDTA, 1% Nonidet (P-40) containing a protease inhibitor mixture (Roche Applied Science, Shanghai, China) and a phosphatase inhibitor mixture (Sigma, MO, USA). An equal amount of total protein was subjected to SDS-PAGE analysis and immunoblotting.

Immunohistochemical staining of HMGB1

Immunohistochemical staining was performed as follows: all paraffin sections were deparaffinized in xylene and rehydrated in a series of graded alcohols. The antigens were retrieved by autoclaving samples in 10mM Tris-HCl buffer (pH 10.0) for 10 mins and cooled to room temperature. After blocking with sheep serum, slides were incubated with mouse antibody against human HMGB1 at a dilution of 1: 500 (BD bioscience, NJ USA) overnight at 4°C. After washing three times with PBS, the slides were incubated with biotinylated secondary antibody at room temperature for 30mins. After washing three times with PBS, slides were stained with the ABC Elite kit (Vectorlabs, CA, USA). Finally, slides were counterstained with hematoxylin, dehydrated, cleared, and then mounted with Permount mounting medium (Fisher Scientific, PA, USA).

In order to evaluate the levels of the expression of HMGB1, immunoreactivity, i.e. both the intensity of staining and the percentage of positive cells (labeling frequency percentage), was estimated by using a 0-4 semi-quantitative system modified from Soumaoro (13) and Peng (14). The staining level of nuclear or cytoplasm was classified into four groups: level of controls (score = 0), weak staining detectable above control (score = 1), moderate staining (score = 2), and deep staining (score = 3). The staining rate was scored as 0 (≤1%), 1 (1%-24%), 2 (25%-49%), 3 (50%-74%), and 4 (≥75%). The total index was a sum of the staining level and rate scores, as follows: (-), (+), (++), and (+++) indicated sum-indexes of 0-1, 2-3, 4-5, and 6-7, respectively; (-) and (+) were defined as no or modest expression, and (++) and (+++) were defined as strong expression. Two pathologists evaluated each section independently and a third pathologist was involved when discrepancy came up.

Statistical Analysis

Results are expressed as means ± standard deviation from at least three separate experiments. A two-sample *t*-test was used to determine statistical differences in the means of two columns. A p-value of less than 0.05 was considered to be statistically significant.

Results

HMGB1 level is increased in both hysteromyoma and endometrial carcinoma. As shown in Table 1, compared with normal endometrial tissue, the levels of HMGB1 are increased in both

hysteromyoma and endometrial carcinoma, as for mRNA levels of HMGB1 (Figure 1). Immunohistochemical staining indicated that the majority of staining for HMGB1 is located in the cytoplasm in endometrial carcinoma while it is in the nucleus in hysteromyoma (Figure 2).

Table 1. Strong expression of HMGB1 in normal, hysteromyoma and endometrial carcinoma tissues

	Total Cases	HMGB1	
		Positive Cases	Positive Percentage (%)
Normal			
Proliferative phase endometrium	20	4	20.0%
Secretary phase endometrium	18	2	11.1%
Hysteromyoma	60	46	76.7%*
Endometrial carcinoma	56	43	76.8%**

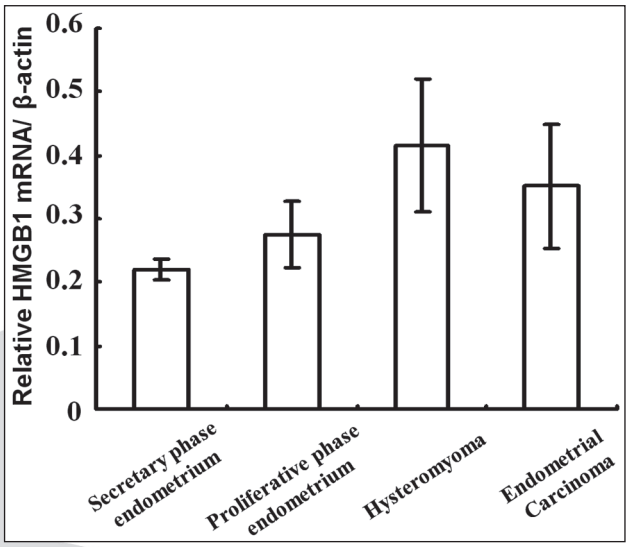

Figure 1. The mRNA levels of HMGB1 in hysteromyoma and endometrial carcinoma tissue are significantly increased compared with normal endometrial tissue

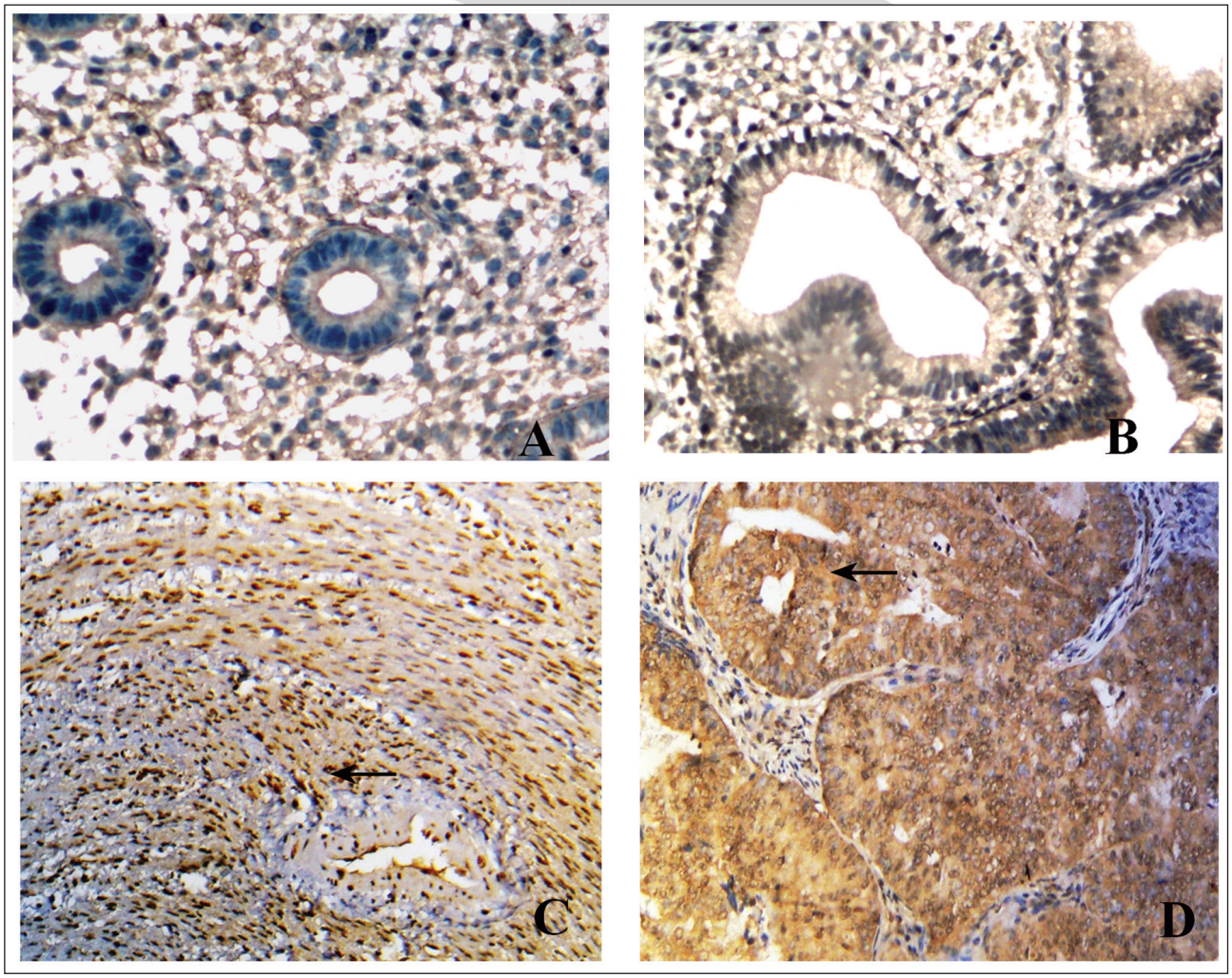

Figure 2. Most HMGB1 in endometrial carcinoma is localized in the cytoplasm, while most of HMGB1 in hysteromyoma is localized in the nucleus

The clinical significance of HMGB1

As shown in Table 2, the HMGB1 expression levels correlate with tumor stages and risk factors. The higher the tumor stage or the greater the risk factors the higher the expression of HMGB1 (for both, $p<0.05$).

Discussion

In order to investigate the expression pattern of HMGB1 in hysteromyoma and endometrial carcinoma, we performed immunohistochemical staining on normal endometrial tissue, hysteromyoma, and endometrial carcinoma and evaluated the relation between HMGB1 expression levels and clinical features of the patients.

In this study, we found that HMGB1 is highly expressed in hysteromyoma and endometrial carcinoma tissues. HMGB1 is a chromatin-binding protein (15,16). HMGB1 is not only an important inflammatory factor but also plays critical roles in tumor growth, invasion and metastasis (2,17). This gene is highly expressed in many types of tumor and involved in several cell signaling pathways related to tumor cell growth, migration and invasion (18-22). It has been reported that the expression of HMGB1 is related to tumor grade, size, and lymphoma metastasis in several types of cancer including pancreatic cancer, lung, and gastric cancer (19-22). In addition, HMGB1 is also an anti-apoptosis protein (23). Overexpression of HMGB1 inhibits cell apoptosis and hence promotes tumor occurrence and growth (2,24). Thus, the elevated expression of HMGB1 in endometrial carcinoma indicates that HMGB1 may contribute to the development of endometrial carcinoma by promoting tumor growth, invasion, and metastasis.

Table 2. Clinicopathologic features of HMGB1 in patients with endometrial carcinoma

	Strong expression n= (%)	**No or modest expression n= (%)**	***P*-value**
Age			0.26
< 50	66.67	33.33	
≥ 50	73.91	26.09	
Menopause			0.0085
Before menopause	77.78	22.22	
After menopause	65.00	35.00	
Tumor grade			
I	79.10	20.90	0.0034
II	78.57	21.43	0.0025
III-IV	77.78	22.22	0.0008
Tumor stage			0.0023
Ia	50.00	50.00	
Ib+II+III	100.00	0	
Lymph node metastasis			0.000174
N0	71.88	28.12	
N1	83.33	16.67	
Differentiation			0.033
Well	68.8	31.20	
Moderately-poorly	83.3	16.7	
Muscle-invasive features			
Non muscle-invasive	58.33	41.67	0.035
Superficial muscle-invasive	76.47	23.53	0.022
Deep muscle-invasive	100.00		
Risks			0.038
Low-risk group	54.5	45.5	
High-risk group	90.9	9.10	

We also observed differences in localization of HMGB1 between hysteromyoma and endometrial carcinoma. In endometrial carcinoma, HMGB1 is primarily localized in cytoplasm. Since cytoplasmic HMGB1 is related to tumor invasion and metastasis, these data indicate that HMGB1 may play an important role in endometrial carcinoma development, invasion, and metastasis. In hysteromyoma, HMGB1 is primarily expressed in the nucleus. Since nuclear HMGB1 can regulate cell growth, elevated levels of HMGB1 in the nucleus in hysteromyoma may primarily promote cell proliferation. Thus, our data suggest that the translocation of HMGB1 from nucleus to cytoplasm may indicate the malignancy of endometrial tissue.

Conclusion

We are the first to demonstrate that HMGB1 is highly expressed in endometrial carcinoma and hysteromyoma, and explore the relation between the clinicopathological features and the expression levels of HMGB1 in endometrial carcinoma. The expression level of HMGB1 is highest in the cytoplasm of endometrial carcinoma, while the expression level of HMGB1 protein is higher in the nucleus in hysteromyoma. These data collectively suggest that cytoplasmic HMGB1 may contribute to development of endometrial carcinoma. Monitoring the expression of HMGB1 in endometrial tissues may help diagnosis or prognosis of endometrial carcinoma.

Authors' contributions

Drs. Zhao, Wu, Zhang, Chen and Yao were responsible for the conception and design of the study, Drs. Zhao and Wu are responsible for the acquisition, analysis and interpretation of data; Dr. Zhao wrote the manuscript. Dr. Wu revised the manuscript critically. All authors read and approved the final version of the manuscript.

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A research on anti-Toxoplasma antibodies in paediatric and adult age groups living in southeast Anatolia

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Abstract

Background: *Toxoplasma gondii* is the infectious agent causing toxoplasmosis. Its prevalence varies depending on geographic location, age, nutrition and hygiene habits. In this study, it was aimed to investigate seroprevelance of antibodies occurring against *T. gondii* in various age groups.

Methods: The presence of toxoplasma IgG and toxoplasma IgM antibodies were searched in female and male cases under and over 18 years who applied to the outpatient clinics of Igdir State Hospital with the suspicion of toxoplasmosis between February and December 2010.

Results: Only one male case <18 years old was anti-Toxoplasma IgM positive 3.5% (n=1/57), whereas all male cases ≥18 years old were anti-Toxoplasma IgM negative (n=9). Six male cases out of 68 <18 years old were anti-Toxoplasma IgG positive 9.8% whereas 2 out of 5 male cases were anti-Toxoplasma IgG positive in group ≥18 years old. In females anti-Toxoplasma IgM positivity rates were 0.09 (n=2/117) and 3.8 (n=50/1328) in under and over 18 years old groups respectively. In addition, in 16.2% (n=20/113) of female cases <18 years old and in 32.4% (n=390/1204) of female cases ≥18 years old anti-Toxoplasma IgG positivity was detected.

Conclusion: Toxoplasmosis is rather prevalent not only in under developed or developing but also in developed populations. Population-based studies without gender discrimination are needed in order to determine the *T. gondii* prevalence accurately.

Key words: Toxoplasmosis, seroprevalance, pediatric, adult, southeast Anatolia.

Introduction

Toxoplasmosis is among the global major zoonotic diseases. It is caused by the obligate intracellular protozoan *Toxoplasma gondii*, with cats as the definitive host, and warm-blooded animals as intermediate hosts. Humans get infections with *T. gondii* after ingesting underdone or raw meat, by ingesting oocysts through contaminated soil, food or water; or congenitally by transplacental migration of tachyzoites (1). Toxoplasmosis is a silent disease generally; however, during pregnancy, may lead miscarriage, stillbirth, preterm birth and congenital abnormalities of the newborn (2). The diagnosis of seronegative women before or in early pregnancy is efficient in preventing the congenital abnormalities. For this reason, the presence of *T. gondii* antibodies ought to be searched for in all pregnant women in their first medical examination (3).

To investigate and underline the importance of toxoplasmosis through determining the seroprevalence of *T. gondii* in women in various age groups whom evaluated with toxoplasmosis pre-diagnosis and besides during routine controls of women in before and after pregnancy, furthermore to evaluate the national and international data, were objectives of this study.

Methods

The anti-Toxoplasma IgG and IgM positivity rates of the patients who applied to Igdir State Hospital between February and December 2010 were determined with Vitros ECI Q J&J Company Ortho-Clinical Diagnostic's macro ELISA apparatus using 3rd generation test kits of the company according to their prospectus. Results evaluated

with chi-square trend analysis according to the age groups and gender. SPSS 17.0 package program was used in statistical assessments.

Results

In the retrospective evaluation of the study population; only one male case <18 years old was anti-Toxoplasma IgM positive (%3.5, n=1/57) (p=0.207), whereas all male cases ≥18 years old were anti-Toxoplasma IgM negative (n=9). Six male cases out of 68 <18 years old were anti-Toxoplasma IgG positive (9.8%) whereas 2 out of 5 male cases were anti-Toxoplasma IgG positive in group ≥18 years old. In females anti-Toxoplasma IgM positivity rates were 0.09 (n=2/117, p=0.207) and 3.8 (n=50/1328, p<0.553) in under and over 18 years old groups respectively. In addition, in 20/113 (16.2%) of female cases <18 years old and in 390/1204 (32.4%) of female cases ≥18 years old (p>0.099 and p=0.717, respectively) anti-Toxoplasma IgG positivity was detected (Tables 1 and 2).

Discussion

The prevalence of toxoplasmosis in the world and in Turkey is between 17.6-76.6 percent. Exploration of anti-Toxoplasma IgG and IgM antibodies to determine their prevalence in the community is important for public health (4).

In this study, we found anti-Toxoplasma IgG positivity in 9.8 % of male and 16.2 % of female (total 26 % of all) cases under 18 years old. In a study from Ankara based on population between 2-12 years, from Kayseri between 0-14 years and in Van between 0-18 years old cases, the IgG positivity rates were found as 13.3, 19.5 and 8.4 percent, respectively (5, 6). In a study in Turkey, *T. gondii* IgM and IgG seropositivity rates were found detected as 1.34 and 24.6, respectively (7). International toxoplasma seroprevalence rates were 37.8 and 8.7 percent respectively in Iran and Korea. However, the seroprevalence rate reported in Korea was lower than in North America, Europa and other Asian countries. This difference was explained with lower cat population in Korea and people living in that country in apartment flats, away from the nature (8, 9). The positivity rate we found in our country was higher than in other countries in the region, albeit lower than in Iran. Although the number of adult male cases was not enough to represent the whole population, there was IgM positivity in 1/57 and 2/117 of subjects, respectively under 18 years old. In addition in subjects <18 years old, IgG positivity rates were lower in males than in females (10).

According to the results obtained in our study, anti-Toxoplasma IgM positivity rate is 3.8 percent for adult females. The same rates were reported from the studies performed with females > 18 years old in various regions of Turkey, while this rate was found 1.4% in a study from Antalya (11). Seroprevalence rates were between 16-40 % and 50-80 % in females for USA and European countries, respectively and 30.1-60.4 % in our country (11, 12). The anti-Toxoplasma IgG positivity in our study was in

Table 1. Distribution the presence of anti-Toxoplasma IgM and IgG antibodies in subjects <18 years old according to gender

Cinsiyet	Anti-Toxoplasma IgM (p=0.207)		Anti-Toxoplasma IgG (p>0.099)	
	Positive	Negative	Positive	Negative
Female	2 (0.9%)	115 (99.1%)	20 (16.2%)	93 (83.8%)
Male	1 (3.5%)	56 (96.5%)	6 (9.8%)	62 (90.2%)
Total	3 (%4.4)	171 (%95.6)	26 (26.0%)	155 (74.0%)

Table 2. Distribution the presence of anti-Toxoplasma IgM and IgG antibodies in subjects ≥18 years old according to gender

Cinsiyet	Anti-Toxoplasma IgM (p<0.553)		Anti-Toxoplasma IgG (p=0.717)	
	Positive	Negative	Positive	Negative
Female	50 (3.8%)	1278 (96.2%)	390 (32.4%)	814 (67.6%)
Male	0 (0.0%)	9 (100%)	2 (40%)	3 (60%)
Total	50 (3.7%)	1287 (96.3%)	392 (32.4%)	817 (67.6%)

this range. Seropositivity rate for adult females is higher than in <18-year age group.

Conclusion

Toxoplasmosis is rather prevalent not only in under developed or developing but also in developed populations. It generally has no clinical signs and symptoms according to our study. This is an important infection in Southern Anatolia as well as all around the world. We think that, it is necessary to draw attention to toxoplasmosis with these studies and diagnostic and preventive methods must be evaluated without overlooking the data obtained in them. Population-based studies without gender discrimination are needed in order to determine the *T. gondii* prevalence accurately.

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An analysis of serum creatin kinase (CK) and lactate dehydrogenase (LDH) concentration of elite handball players during a tournament

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Abstract

Background: It is known that regular and medium intensity exercises have various positive effects on the health. However some forms of the exercise types and especially maximal or acute high intensity exercises may cause muscle injuries by increasing the secretion of the CK and LDH skeletal muscle enzymes.

Purpose: The purpose of the study has been determining the changes taken place in CK enzyme activity and LCH concentration values of the handball players before and after a tournament.

Method & materials: Twelve elite handball players at the age of 22.16±1.85, 181.16±5.18 in height and 81.59±11.99 in body weight have participated in the study on a voluntary basis. The serum CK and LDH levels of the subjects have been measured before and after the tournament.

Results: It has been found out that after the tournament both the CK and LHD concentration levels of the handball players have significantly decreased in statistical terms in comparison to the pre-tournament measurement (p=0.031, p=0.001).

Conclusions: It can be claimed that statistically significant decrease of the CK enzyme activity measured after the handball game indicates that there is no or very little risk of obtuse injury stemming from muscle injury of the handball players. The study needs to be supported by the studies conducted with more subjects and in various branches.

Key words: Creatine kinase, handball, tournament, muscle injury.

Introduction

The handball is performed professionally in mainly Europe and all around the world as an Olympic game. However in comparison with the other sports, there are very few studies conducted on handball players. The handball is a dynamic sport that has aerobic and metabolic demands. During handball tournaments the players may get injured due to effects such as muscle fatigue, contact and collision. Especially, 3-4 games in one week during a tournament may cause the players to be exhausted, to have muscle injuries and inflammation (Castagna et al. 2009). It is known that regular and medium intensity exercises have various positive effects on the health (ACSM, 1998; Shephard et al. 1998; Balci et al. 2012). However some forms of the exercise types and especially maximal or acute high intensity exercises may cause muscle injuries by increasing the secretion of the CK and LDH skeletal muscle enzymes (Nivaldo et al. 2012). The muscle injury is determined by observing the changes taking place in CK and LDH concentrations in the blood three hours after the exercise (Paschalis et al. 2007; Chatzinikolaou et al. 2010; Hammouda et al. 2011; Rodrigues et al. 2010). CK and LDH are the enzymes in cytosol and mitochondria of the tissues (Schlattner et al. 2006; Baird et al. 2012; Nivaldo et al. 2012). CK is known as the center of the energy network in phosphocreatine cycle. In that cycle, cytosol enzymes provide ATP production for muscle activity (Saks 2008). Therefore, increase in the serum CK level is quite closely related with the muscle injuries. Measuring serum CK levels is an important indicator in determining the isoenzyme profiles (Brancaccio et al. 2007; Totsuka et al. 2002). Reilly and Ekblom have stated that CK level may cause muscle injury after 24 and 72 hours from the exercise depending on age and exercise habits of the individuals (Reilly and Ekblom 2005).

Takahashi et al. have emphasized that there has been significant increase in muscle enzyme activity of rugby players after the games (Takahashi et al. 2007). In the literature there are plenty of studies on the effects of the breaks after the power exercises or during power trainings on CK and LDH levels (Sima˜o et al. 2007; Rodrigues et al. 2010; Jamurtas et al. 2005; Paschalis et al. 2007). Nivaldi et al. have found out that there is significant increase in the CK and LDH enzyme levels of the sportsmen after a football game (Nivaldo et al. 2012). However, there is no study on CK and LDH concentrations of the handball players before and after a game. In addition, the examples in the literature only cover measurements following a game or loading period. This study, on the other hand, aims at determining the CK and LDH statuses of the handball players before the game (BG) and after the game (AG) when they play games on successive days like in the tournaments. With this aspect, the study will contribute to the literature and it is original since it studies the BG and AG values. In this context, the study aims at determining the changes in the CK and LDH values of the handball players before and after the tournament.

Material and Method

Twelve elite handball players at the age of 22.16±1.85, 181.16±5.18 in height and 81.59±11.99 in body weight have participated in the study on a voluntary basis. The criteria for participation: (a) looking physically healthy, (b) not using medicines or not having any medical obstacle to participate in the study (c) not using any supplementary food that boosts the performance (such as creatin). Participant handball players have played games in single period league method for a period of five days. All the participants have been informed about the study, the consent forms have been read and signed. Experimental protocols have been confirmed by Kayseri Clinical Research Ethics Committee. The ages, heights, body weights, body mass index (BMI) and body fat rates, serum CK and LDH levels of the subjects have been measured. In determination of the ages of the sportsmen Identity Card information has been used. Their heights have been measured with a meter and the body weights have been measured with an electronic scale and recorded in cm and kg respectively. The formula (body weight (kg) / body height (m^2)) has been used for BMI (Eston and Reilly, 2009), for body fat rate (BFR) has been used Green Formula (BFR = 3,64 + Total Skinfold* x 0,097) (Green, 1970). *[Total Skinfold: Skinfold measures taken from biceps, triceps, subscapular, subrailiac, abdominal and femoral areas with skinfold caliper (Eston ve Reilly, 2009)].

The sportsmen have been warned about not eating or drinking anything after 10 pm before their blood samples have been taken. The blood samples have been taken at 9-10 am in the laboratory. 5 ml blood samples have been taken BG and AG from the left forearm antecubital area of the sportsmen in sitting position in line with the hygiene rules into tubes with and yellow lid. Serum Creatin Kinase (CK) and Lactate Dehydrogenase (LDH) levels have been determined in spectrophotometer (Spectro Max pro 4.6.) and at the wave length of 340 nm in line with the kit (Spinreact, S.A) procedures.

SPSS program has been used in analysis of the data. The results of the measurements have been presented as the mean and standard deviation. Normality analysis of the data has been tested with *"Shapiro Wilk"* and *"Kolmogorov Smirnov"*. As the data have demonstrated a normal distribution, "Student-t test" has been applied in dependent groups in comparison of the BG and AG values and significance level has been recognized as $p<0.05$.

Findings

Physical measurement values of the handball players are presented at the table 1.

Table 1. BG and AG Physical Measurement Values

Parameters (n=12)	**X±Sd**
Age (year)	22,16±1,85
Height (cm)	181,16±5,18
Body Weight (kg)	81,59±11,99
BMI (kg/m^2)	24,81±3,07
BFR (%)	12,08±2,99

Table 1 demonstrates the physical measurement values of the sportsmen. BG and AG CK enzyme activities and LDH concentrations are presented at the table 2.

It is seen in Table 2 that both CK enzyme activity and LDH concentration values decrease significantly in favor of AG statistically ($p<0.05$). BG

Table 2. CK and LDH Values of the Subjects

Parameters (n=12)		X	t	p
CK	Pre-tournament	15,08±3,65	2.475	0.031*
	Post-tournament	12,00±4,32		
LDH	Pre-tournament	207,75±25,78	4.388	0.001*
	Post-tournament	174,50±33,17		

*(CK: Creatin kinase, LDH: Lactate dehydrogenase, *p<0.05)*

CK values of the sportsmen were 15.08±3.65, and AG the values were 12.00±4.32. This change is statistically significant (p=0.031). In terms of LDH concentration, BG values were 207.75±25.78 and AG the values decreased down to 174.50±33.17. This decrease is statistically significant (p=0.001).

Discussion

Measuring muscle enzyme activity after high intensity exercises is used for estimating possible muscle injuries after sports activities. Exercise based possible muscle injuries can be determined by measured muscle enzyme activity (Apple and Rhodes 1988). In the study, the changes taken place in CK and LDH enzyme activities of the handball players before and after the tournament have been analyzed. Measurement of CK and LDH concentrations of the handball players before the tournament demonstrated statistically significant decrease in both CK and LDH concentration values after the tournament (p=0.031, p=0.001). Some researches in the literature have found out that high intensity and long period exercises increase the muscle enzyme secretion and cause muscle injury (Koutedakis et al. 1993; Smart et al. 2008; Brancaccio et al. 2010). However, it has been reported that similarly muscle enzyme secretion may take place after an acute exercise or game (Finaud et al. 2006; Petibois et al. 2001). CK is an enzyme in the blood that used in assessing the muscle injury. Increasing CK activity secretion after acute exercises is in dispute. Some studies have claimed that the highest secretion time of CK level is right after acute exercises (Brancaccio et al. 2007), while some others have claimed that it is after 24-36 hours (McLellan et al. 2011; Takara 2003). In the literature it has been claimed that both the CK and LDH concentrations of the subjects which perform certain resistance exercises increase following the exercise and stay high even after 24-48 hours and only go down to normal levels after 72 hours (Ferri et al. 2006; Jamurtas et al. 2005; Rawson et al. 2007). In their study on professional rugby players, McLellan et al., have found out that CK levels of the sportsmen increase right after a rugby game and after 24 hours this increase has reached to the highest level (McLellan et al. 2011). Plazma CK enzyme activity may cause obtuse injuries that as a physical result of the increases taken place (Hoffman et al. 2002; Strojnik and Komi 1998). In the study, on the other hand, it can be claimed that measured CK enzyme activities following the handball game have significantly increased and therefore there is no or very little risk for obtuse injury due to muscle injury. McLellan et al., have claimed that sportsmen may have the risk of obtuse injury due to muscle injury as a result of increased serum CK enzyme activity after a rugby game. (Mc Lellan et al. 2011). In the study, contrary to the literature, it has been found out that CK levels of the handball players significantly decrease at the end of the tournament. In the literature, single games or high intensity exercises have been analyzed and increases have been observed in LDH and CK values (McLellan et al. 2011; Brancaccio et al. 2007; Hoffman et al. 2002). However, in this study significant decrease has been detected in the CK enzyme activity and LDH concentration of the handball players who played 4 games in a handball tournament for 5 days (p=0.031, p=0.001). It can be claimed that the difference between the literature and the study stems from the fact that the study has followed a tournament process.

Conclusion

For better evaluation of the findings, CK enzyme activity and LDH concentrations of the sportsmen in team or individual sports should be analyzed on the basis of tournaments. Because in

the literature, the studies have been conducted only on single games (McLellan et al. 2011; Takarada 2003). However, across the globe sportsmen play games on successive days without any breaks. Therefore, it is thought that following the process for CK enzyme activity and LDH concentrations before and after the tournament would make more sense. It is only a claim that acute changes in CK and LDH values of sportsmen may cause muscle injuries. If these acute changes were to cause obtuse injuries due to muscle injuries for sportsmen, probably many sportsmen would be affected in a negative way. Moreover, after every tournament, exercise or competition sportsmen could get injured. However it can be observed that such a situation is not likely to take place. However, determining the levels of CK enzyme activity and LDH concentration changes of the sportsmen at the end of a tournament may make more sense for being able to discuss muscle injuries. From this point of view, the study should be supported by other studies conducted on more subjects who are from various branches in this field.

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Determination of the relationship between the level of autonomy and job satisfaction of nurses and the sociodemographic factors that affect them

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Abstract

Aims : This research is a study made in order to determine nurses' autonomy and level of job satisfaction which are affected by socio-demographic factors, occupational characteristics, the relationship between autonomy and job satisfaction.

Background: The personnel with a higher level of job satisfaction and autonomy are more motivated about their jobs and provide a higher quality service.

Materials and Methods: The research is applied on 178 nurses working at a public hospital in the northeastern part of Turkey. Research data is obtained by socio-demographic questionnaire, Minnesota Job Satisfaction Scale and the autonomy part of Sociotropy-Autonomy scale. Evaluation of the data is done via computer percentage, mean, t-test and correlation analysis.

Results: It is found that, 50.6% of nurses participating in the study having the bachelor's degree, while 93.3% of them working as nurse-in-service and 84.4% of married. In addition, it is found that, the average age of nurses is 36.6 ± 6.1 and average years of work experience is 16.6 ± 6.4. Job satisfaction scores, average scores of autonomy found as 60.6 ± 12.2, 70.31 ± 17.4 respectively. While a significant difference was found between their educational status and average scores of autonomy, such a good relation wasn't not found between the levels of job satisfaction and autonomy.

Conclusions*:* Nurses should be made a part of all decision making processes concerning the improvement of health care standards and the practice of nursing services with a view to increasing their autonomy and job satisfaction.

Key words: Autonomy, job satisfaction, nurse.

Introduction

Today, in the health care system the role of nurses becomes more complicated and their responsibilities increase every day. Among these changes are the nursing shortage, the varying length of hospital stay, cost effectiveness, shrinking health care facilities, and increasing number of acute and chronic illnesses. All these changes increase the need for autonomy in nursing practices (1).

Autonomy is a multifaceted phenomenon. The word is derived from the Greek words *auto* (self) and *nomos* (law). It signifies the governing of oneself and gives the health care personnel the right to approach patients every way that lies within the liberty defined for the nursing practice socially and legally and to make independent health care-related decisions (2). According to Steward, an autonomous nurse should be knowledgeable about and free in making decisions which he or she thinks would benefit the people he or she is responsible for, concerning issues that fall within his or her domain of competence (3).

Many studies show that nurses do not have sufficient autonomy (1,2,4,5,6). Very rarely were nurses shown to have a high level of autonomy (7).

Besides being important for the independence of the personnel, autonomy is also an important factor affecting job satisfaction. Previous studies show that autonomy is the most decisive indicator of job satisfaction and productivity, and that low levels of autonomy and job satisfaction among nurses lead to disappointment, professional dissatisfaction, exhaustion, and lowered control at the workplace (2,8, 9). Many studies indicate that there is a positive relationship between autonomy and job satisfaction, and that higher levels of au-

tonomy lead to higher levels of job satisfaction. In a study conducted by Zurmehly *et al.,(9)* it was found that there was a strong positive relationship between autonomy and job satisfaction, and that the level of job satisfaction among nurses increased as the level of autonomy went up (9). In their study, Iliopoulou *et al.,* also had findings that supports those of Zurmehly *et al.'s* (2,9).

Job satisfaction is a positive factor that links the personnel to their jobs. The personnel with a higher level of job satisfaction are more motivated about their jobs and provide a higher quality service. When the health care personnel are content with their own situation at the work place, they concentrate their energy on improving the quality of health care services (10,11). Therefore, job satisfaction among nurses plays an important role in increasing their productivity and efficiency and in increasing the quality of health care services provided to patients.

No previous study was found on the relationship between the level of job satisfaction and autonomy in Turkey. It is necessary for nurses to take control of situations that affect themselves and patients receiving health care and to act autonomously in order for the nursing to be accepted as a professional occupation.

Aim

This is a descriptive and correlational research that aims to determine the level of autonomy and job satisfaction among nurses, their affecting factors as well as the relationship between the two.

Methods

Type of the research

This is a descriptive and correlational research.

Location

This study was conducted on the nurses working in internal diseases, surgery and intensive care units of a state hospital between January and April 2012.

The population and the sample of the study

The population of the study consists of 312 nurses working in internal diseases, surgery, and intensive care units of a public hospital in hospital. No further sampling was performed. However, those who were on sick leave or leave of absence at the time of the research, those who did not want to take part in the research, and those who did not fill in the forms appropriately were not included in the research so the research was completed with a total of 178 nurses. This number makes 55% of the whole population.

Tools of data collection

Three kinds of questionnaire forms were used to collect the necessary data. The first was the questionaire form to determine the characteristics a descriptive assessment form prepared in light of the existing literature with a view to assessing the demographical characteristics of the nurses working in the hospital. The second and the third were the Minnesota Job Satisfaction Scale and the Sociotropy-Autonomy Scale respectively. Sociotherapy-authonomy scale is the one consisting of two parts and in this study only the authonomy part was used. The permition to use the only authonomy part of the scale was taken from the author.

Socio-demographic questionnaire form consists of 9 closed end questions, such as concerning the nurses' age, educational status, current position, total term of employment, term of employment within current unit, marital status, and opinions about the nursing profession as well as the publications subscribed and professional organizations joined by them. The questionnaire form was prepared with contribution of the literatüre (6, 7,12,13).

The Minnesota Job Satisfaction Scale (MSQ) is a form that consists of 20 questions responded on a 5-point Likert Scale, which was developed by Weiss, Dawis, England & Lofquist (1967) (14). The validity and reliability of the Turkish version of the scale (Cronbach Alpha = 0.77) was conducted by Baycan (1985). Every question comes with five alternative responses indicating the level of satisfaction of the participant(15). The possible responses are: "Very Dissatisfied", "Dissatisfied", "Neither", "Satisfied", and "Very Satisfied". These responses are assigned 1, 2, 3, 4, 5 points respectively. In this scale, the highest possible score is 100, while the lowest possible score is 20. A score of 60, which is the midpoint in the range, represents neutral satisfaction. Scores that are closer to 20 indicate a low level of satisfaction, while scores closer to 100 mean a higher level of job satisfaction.

The Sociotropy-Autonomy Scale (SAS) was developed by Beck et al. and adapted to Turkish by Sahin *et al*., (14). Half of the items in the scale belong to the sociotropy scale, while the remaining half belongs to the autonomy scale. Every question is responded on a 5-point Likert scale on the basis of how well it describes the responding participant, varying from "It does not describe me at all" to "It describes me very well". The questions of the two scales are presented in no specific order. The highest possible score on each scale is 120 points. High scores on the sociotropy scale indicate a sociotropic personal character; while high scores on the autonomy scale suggests an autonomous character. A participant's score on the autonomy sub-scale is calculated by summing his or her scores on the 12-item Individual Achievement Autonomy Sub-Factor (ASF) which is a precondition for autonomy (2, 3, 9, 12, 14, 20, 30, 32, 39, 45, 48, 60), the 12-item Independence ASF which facilitates independent decision-making (6, 13, 21, 22, 23, 28, 36, 41, 43, 54, 55, 57), and the 6-item Preference for Solitude ASF which reflects the ability to be independent and self-sufficient (10, 16, 25, 37, 42, 51).

Collection of Data

The data collection forms were distributed to all the nurses in the hospital with the help of nursing directors and the nurses in charge of the units included in the study. The nurses were asked to fill in the forms within the specified time frame. It was ensured that the forms were handed in to the nurses in charge of the units in a closed envelope, who then submitted all the envelopes to the researchers.

Dependent and Independent Variables

Age, educational status, current position, total term of employment, term of employment within current unit, professional publications subscribed, membership status to professional associations, marital status, and opinions about the nursing profession constitute the independent variables of this research, while the level of autonomy and job satisfaction are the dependent variables.

Analiysis

The data were evaluated using the SPSS 16 software package. The demographical data was statistically analyzed by calculating numbers, percentage distribution, and mean values. As for the factors affecting autonomy and job satisfaction, the t-test and Anova analysis were employed. The Pearson Correlation analysis was performed to evaluate the relationship between the two.

Ethical Considerations

The necessary permissions were obtained both from the hospital management and the city health administrative. The aim of the research and the researchers' expectations of it were orally explained to the participants. The participants were also assured that their identities would be kept confidential, and that their information would be used exclusively in this particular research. Only those who volunteered and filled in the necessary forms appropriately were included in the study. Forms with missing information were not included in the analysis.

Results

The mean age of the participating nurses was found to be 36.67±6.13. Majority of them (% 48.9) were in the 34-40 age group; 84.8% were married; and 50.6% held a college degree. Majority of the nurses had a term of employment of between 14-21 years (44.4%). 29.3% of the participants were working in the interneal diseases unit, while 65.1% and 5.6% of them were working in the surgery and intensive care units respectively. 92.7% of the nurses were working as a charge nurse. It was found that 75.3% of the nurses were not a member of any professional associations, and that 89.3% did not follow any scientific publications. When they were asked about their opinions about their profession, 51.7% were found to have positive opinions about nursing; 21.3% held negative opinions; and 27% were undecided.

The autonomy scores of the participants are shown in Table 1. The general mean autonomy score was found to be 70.3±17.4. As for the mean scores of the subscales of the autonomy scale, the mean score was 30.8±7.8 for the Individual Achievement Autonomy Sub-Factor (ASF) which is a precondition for autonomy, 26.9±7.2 for the Independence ASF which facilitates independent decision-making, and 12.5±5.2 for the Preference for Solitude ASF which reflects the ability to be independent and self-sufficient. These mean scores indicate a character that is more auotonomous than avarage.

Table 1. The mean autonomy scores of the participants

	Minimum	Maximum	X±sd
Individual Achievement	11.0	48.0	30.8±7.8
Independence	8.0	46.0	26.9±7.2
Preference for Solitude	2.0	24.0	12.5±5.2
General Autonomy	**21.0**	**114.0**	**70.3±17.4**

Table 2. The mean job satisfaction scores of the participants

	Minimum	Maximum	X±sd
Internal Satisfaction	16	60	38.8±7.4
External Satisfaction	8	40	21.8±5.7
General Job Satisfaction	**28.0**	**100.0**	**60.6±12.2**

Table 3. The mean autonomy and job satisfaction scores according to the demographic and professional characteristics of the nurses

Demographic Characteristics	Number	%	Autonomy	F, p	Job Satisfaction	F, p
Educational level						
Health High school	12	6.7	75.0±13.6		65.2±16.3	
Associate Degree	66	37.1	71.0±18.0	**F=3.52, P<0.05**	61.5±12.0	**F=0.98, P=0.40**
Baccalaureate	90	50.6	67.5±16.7		59.5±11.8	
Masters	10	5.7	84.8±16.4		59.3±11.9	
Age						
20-26	6	3.4	70.8±16.0		52.3±5.7	
27-33	47	26.4	70.1±16.6	**F=0.97, P=0.406**	59.8±11.3	**F=2.30, P=0.07**
34-40	87	48.9	68.5±17.8		60.0±11.6	
41-↑	38	21.3	74.3±17.7		64.4±14.6	
Years of experience						
10 year and ↓	33	18.5	63.3±16.4		58.6±10.8	
11-16 year	58	32.6	73.8±17.1	**F=3.30, P<0.05**	56.7±12.0	**F=3.29, p<0.05**
17-22 year	57	32.8	68.7±16.0		62.0±10.5	
23 year and ↑	30	16.9	74.0±19.6		64.2±15.1	
Present Position						
Clinical Nurse	165	92.7	70.4±17.8	**t=0.26**	60.3±12.2	**t=1.36**
Administrator Nurse	13	7.3	69.0±10.4	**p>0.05**	65.± 12.4	**p>0.05**
Marital status						
Married	151	84.8	68.9±17.1	**t=2.46**	61.1±12.2	**t=1.18**
Single	27	15.2	77.8±17.3	**p>0.05**	58.1±12.5	**p>0.05**
Professional thinking						
Positive	92	51.7	73.0±17.0		65.5±11.5	
Negative	38	21.3	72.7±19.8	**F=5.72, P<0.05**	50.5±8.9	**F=26.2 P<0.01**
Undecided	48	27.0	63.2±14.4		59.3±10.6	
A member of the association						
Yes	44	24.7	69.8±19.9	**t=2.68**	59.2±10.6	**t=1.92**
No	134	75.3	70.4±16.5	**p>0.05**	61.1±12.7	**p>0.05**
Followed by professional publication						
Yes	19	10.7	72.1±14.1	**t=0.48**	67.7±10.8	**t=2.70**
No	159	89.3	70.0±17.4	**p>0.05**	59.8±12.1	**p<0.05**
Work area						
Internal units	52	29.3	68.0±16.9		56.5±11.2	
Surgical units	116	65.1	71.7±17.4	**F=1.29 p>0.05**	63.1±11.9	**F=7.12 p>0.05**
Intensive Care Units	10	5.6	65.2±19.8		54.1±13.7	

The mean job satisfaction scores of the participants are shown in Table 2. It was found that the nurses had a mean job satisfaction score of 60.6±12.2. Considering the minimum and maximum possible scores for this scale, it can be suggested that the mean job satisfaction of the nurses indicate a "moderate level" of job satisfaction.

The distribution of the participants' autonomy and job satisfaction scores according to their demographic and professional characteristics is shown in Table 3. These figures suggest that there is no statistically significant difference between their mean autonomy and job satisfaction scores according to their age, marital status, unit of service, current duty, association memberships, and professional publications subscribed ($p>0.05$). A statistically significant difference was found between the mean autonomy and job satisfaction scores according to term of employment and the opinions held about the profession ($p<0.05$), while no statistical difference was identified between job satisfaction and educational status.

Table 4. The relation between mean job satisfaction scores and mean authonomy scores of the nurses who took part in study

Variables	N	r	P
Job Satisfaction Autonomy	176	0.012	0.870

Table 4 shows that there's no significiant relation between the nurses' mean job satisfaction and mean autonomy scores ($p>0.05$).

Discussion

This study has found nurses' mean autonomy scores to be 70.3±17.4 in personal achievement 30.8±7.8, independence 26.9±7.2, and preference for solitude respectively 12.5±5.2. When these figures are considered on the basis of the minimum and maximum scores for the scale, the nurses can be said to express a moderate level of autonomy. In a study conducted by Kangalli and Karagozoglu(4) in order to determine the level of autonomy among nurses and some of the affecting institutional factors, the participating nurses were found to have a mean autonomy score of 75.95±16.22. Like our study, their study also found that the nurses showed a moderate level of autonomy. Autonomy, as a distinctive feature of professionalism, has historically developed less within the profession of nursing compared to other professions (16). In their respective studies, Iliopoulu and White (2) and Fung-Kam (6) found that nurses had a low level of autonomy. In a study found high levels of autonomy among nurses (7).

A statistically significant difference was found between the general mean autonomy scores varying on the basis of educational status ($p<0.05$), and the level of autonomy was found to increase those whose educational status are high. Much as there are many studies in the literature showing a positive relationship between educational status and autonomy (1,17,18,19), there are also many studies showing that educational status has no effect on autonomy (7,9). Erdoğan and Akyolcu(20) found that the nurses were more capable of making decisions independently as their level of education increased. Papathanasoglou *et al.* (21),similarly found that the nurses with a college degree had a higher level of autonomy in decision making processes. In their study, Karagozoglu and Kangalli(4) did not find a statistically significant difference between the general autonomy mean sores and mean autonomy sub-scales scores varying on the basis of level of education ($p> 0.05$); however, they found a positive relationship between the level of education and the level of autonomy. It is seen that nursing education affected the authonomy Taking responsibility and making independent decisions are skills that should be developed and reinforced over the course of nursing education. The lack of a standard in the nursing education in Turkey, that is, the presence of nurses with a high school degree, a vocational high school degree, and a college degree, is shown as one of the most important factors preventing the development of autonomy in the nursing profession (22). Therefore, nursing education should be based on a college degree.

In this study, the mean job satisfaction score among the participants was found to be 60.6±12.2 (Table 2). When this figure is evaluated in light of the maximum and minimum scores for this scale, it can be said that the mean job satisfaction scores among the participating nurses express a "moderate level" of job satisfaction. In their respective studies, Golbasi *et al.,* (23), Abualrub & Alghamdi (24),

Lorber & Savic (25), Celik & Hisar (12) also found the nurses to have a "moderate level" of job satisfaction. The findings of these studies cohere with those of ours. Nurses often face job dissatisfaction because of irregular and heavy working conditions, lack of sleep, fatigue, lack of precision in the descriptions of duty, authority, and responsibilities, unequal pay, professional status, and serving to people who are in pain or dying (26). Ineffective ways of coping with occupational stress in nursing profession might bring about organizational consequences such as negativity towards profession, indifference, constant complaining, criticism, absenteeism, and quitting (27). These consequences might affect the quality of the comprehensive patient care to be provided and decrease job performance. Many previous studies present results justifying these predictions and find the nurses to have a low level of job satisfaction (28).

When the mean autonomy and job satisfaction scores were considered on the basis of the nurses' term of employment, it was found that both the mean autonomy and job satisfaction scores went up as the term of employment increased. Studies that focus on the impact of term of employment on job satisfaction present varying results on the relationship between total term of employment and job satisfaction and autonomy. Much as there are studies that suggest a very close relationship between term of employment and job satisfaction and autonomy (6, 12, 24, 25, 26), there are also studies that show a negative relationship between term of employment and job satisfaction (29).The increase in job satisfaction that comes with higher terms of employment can be explained by some outcomes of higher terms of employment such as increasing expertise, complete internalization of role and responsibilities, a higher level of importance attached to experience in one's team, decreasing number of night shifts, and growing appreciation of one's opinions by the administration. However, there are also studies suggesting that the term of employment does not affect job satisfaction (23, 28, 30, 31).

A statistically significant the difference was identified between the participants' opinions about their profession and their mean job satisfaction and autonomy scores ($p<0.05$). Those who held positive opinions about their profession had high mean job satisfaction and autonomy scores, while those who had negative opinions had lower scores. In a study conducted by Keskin & Yildirim (32), the participating nurses' job satisfaction and professional satisfaction were analyzed. It was found that the nurses who were content with their profession had higher mean job satisfaction job satisfaction scores than those who were not content. In a study conducted by Kocaman & Gok(27), it was found that 67.2% of the nurses were dissatisfied with their work conditions. They also found that 43.3% of the nurses quit their profession because of their negative perceptions of the nursing profession. In a study conducted by Kahraman *et al.,(33)* it was found that 67.5% of the nurses did not choose their profession willfully, and only 5.6% found nursing to be suitable for themselves. They also found that 47.2% of the nurses were optimistic about the future of nursing. Finding the profession to be suitable for oneself and having positive opinions about the profession indicate job satisfaction. Job satisfaction is a positive factor that links to personnel to their jobs. The personnel with a higher level of job satisfaction are more motivated about their jobs and provide a higher quality service. When the health care personnel are content with their own situation at the work place, they concentrate their energy on improving the quality of health care services. Therefore, job satisfaction among nurses plays an important role in increasing their productivity and efficiency and in increasing the quality of health care services provided to patients.

The high rate of those with a positive opinion about the nursing profession can be attributed to the fact that 56.3% of the sample held either a college or graduate degree, and that the number of nurses with a term of employment of below 10 years were rather low. In fact, the nurses in the first years of their career tend to have more negative opinions about their profession because of adaptation problems, excessive work load, not having the benefits that come with seniority, relatively lower duty status, and relatively lower income.

According to the results of the correlation analysis between the mean job satisfaction and autonomy scores among the participating nurses, there was no statistically significant relationship between the mean job satisfaction and autonomy scores ($p>.05$). The findings of Fung-Kam's study (6) resemble with those of ours. Other studies emphasize the presence of a strong relationship be-

tween autonomy and job satisfaction (2,9,13,34). It is thought that the findings of our study diverge from those studies because the sample had different characteristics and the tool measuring professional autonomy was not used in our study.

Conclusion

In this study conducted to determine the relation between the nurses' authonoym and their job satisfaction levels and the factors affecting them, and authonoym and job satisfaction, it was discovered that the nurses' mean job satisfaction and mean autonomy scores are in medium level. Moreover, between nurses' years of work experience, their opinions concerning the profession and their mean job satisfaction and mean autonomy scores as well as their educational status and mean authonomy scores was found a significant correlation, while such a relation wasn't between job satisfaction and mean authonomy.

In light of these results, the following points are recommended:

- Nursing education should be based on a college degree.
- Nursing education curricula should be designed to improve autonomy among nurses and to include such skills as critical thinking, problem solving, decision making, assuming responsibility, self-evaluation, communication, and leadership.
- Nurses should be given the organizational authority and responsibility that would encourage them to make decisions independently.
- Nurses should be made a part of all decision making processes concerning the improvement of health care standards and the practice of nursing services with a view to increasing their autonomy and job satisfaction. It is also recommended that this study be conducted with a bigger sample and using instruments measuring professional autonomy.

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Does body perception affect the level of social communication?

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Abstract

Objectives: The Body Esteem of the subjects may affect different behaviours, such as social communication skills.The relationship between Social Communication Skills Rating Scale (Adult Form-Public Interaction Skills) (SCSRS) and The Body Esteem Scales (BES) of the university students in different faculties was investigated.

Methods: 300 students, choosed randomly from the faculties of Anadolu University and Eskişehir Osmangazi University, were included into the study. They asked to fill the Questionnaire, BES and SCSRS. The students of Fine Arts Faculty had higher BES and SCSRS values compare to the Faculty of Medicine and Faculty of Communication Sciences.

Results: The students, happy with their lips, ears, width of shoulders, figure or physique, appearance of stomach, generally had higher SCSRS scores. Whereas higher muscular strength, agility, higher energy levels or higher sex activities were related to lower social communication results.

Conclusions: In Turkish population, facial beauty is very important for most of the subjects; and it may cause to greater self-confidence. In university students, social communication skills were affected by Body Esteem Scales of them. From my childhood upbringing, social norms, view of sexuality, cultural structure of society for the perception of the shape and beauty influence the perception of the body, and this changes their social communication skills.

Key words: Social Communication Skills Rating Scale (Adult Form-Public Interaction Skills) (SCSRS), The Body Esteem Scales (BES), university students.

Introduction

Social communication also involves both verbal and nonverbal components. Good communication ability plays a fundamental role in making and keeping friends. A child or adult with well-developed social communication skills incorporates the needs of conversational partner(s), situational and emotional factors, appropriate language rules, and social mores, and can shift the level of language necessary based on the demands of the moment. These are complex skills that most children learn by the time they are entering school (1).

Body language is a form of mental and physical ability of human non-verbal communication, consisting of body posture, gestures, facial expressions, and eye movements. Humans send and interpret such signals almost entirely subconsciously (2). It is stated that human communication consists of 93 percent body language and paralinguistic cues, while only 7% of communication consists of words themselves (3).

Effective communication skills may facilitate the relationships in all kinds of human and in the professional relationships. Professionals must have communication skills, particularly if working in the areas communicated to the humans., such as psychiatrist, psychologist, counselor, social worker, physician, nurse, teacher, banker, lawyer or work as a salesperson (4). Communication skills are thought to be gained by Congenital way and/or intuition, whereas studies show that most of the techniques have teachable and learnable features (5,6).

In the present study, we investigated the relationship between Social Communication skills and Body Esteem Scales (BES) (7-9) of the university students. The research was conducted in the students of Faculty of Medicine (FM), Faculty of

Fine Arts (FFA) and Faculty of Communication Sciences (FCS).

Materials and method

This prospective study was conducted in Faculty of Medicine, Eskişehir Osmangazi University; and Faculties of Fine Arts, and Communication Sciences, Anadolu University.

Subjects

Three hundred students, choosed randomly from three faculties were included into the study. Students from Faculty of Medicine (n=100) (52 male, 48 female), Faculty of Fine Arts (n=100) (50 male, 50 female) and Faculty of Communication Sciences (n=100) (64 male, 36 female) were asked if the accepted to participate the study; and to fill the questionnaire. Questionnaires in this study was filled after the subjects were verbally informed and they accepted to enter the study by verbal consent of them. 271 students were between 20-25 years-old, 22 students were between 26-30 years old; and 7 students were between 30-35 years old group.

Instrumentation

Questionnaire: During the research, a questionnaire was used to investigate the socio-demographic characteristics of individuals [Age, gender, which children in a family, Graduaded Secondary School, Monthly income, Live place before university, Regular Sex (Yes, No), Bad habits (Smoking and/or alcohol usage)]. The data was collected using face to face interview technique.

The Body Esteem Scale (1-3) (BES): The Body Esteem Scale was developed by Fronzoi & Shields (1984) (7-9). The scale includes 35 pieces of body parts and functions. Students were asked to indicate how they feel about those part or functions of their own body using the scale. Students filled scale according to their personal options on each items. Likert-type scale was used. “Have strong positive feelings” 5 points, “Have moderate positive feelings “ 4 points “Have no feeling one way or the other” 3 points, “Have moderate negative feelings” 2 points “Have strong negative feelings” numbered to 1 point.

The items of Body Esteem Scale: Body scent (BS); Appetite (Ap); nose (N); Physical stamina (PS); Reflexes (R); Lips (L); Muscular strength (MS); Waist (Wa); Energy level (EL); Thighs (T); Ears (E); Biceps (B); Chin (C); Body build (BB); Physical coordination (PC); Agility (Ag); Width of shoulders (WS); Arms (Ar); Chest or breasts (CB); Appearance of eyes (AE); Cheeks/cheekbones (CC); Hips (Hi); Legs (Le); Figure or physique (FP); Sex drive (SD); Feet (F); Sex organs (SO); Appearance of stomach (AS); Health (He); Sex activities (SA); Body hair (BH); Physical condition (PCo); Face (F); and Weight (We).

Social Communication Skills Rating Scale (Adult Form-Public Interaction Skills) (SCSRS) (10): SCSRS was used for rating the social communication skills. The adult form scale was used: 1 shows Rarely uses abilities, 2 shows Sometimes uses abilities, 3 shows Almost always uses abilities. There were 15 items of SCSRS:

1 Eye contact (EC): Looks at to others while talking and listening.
2 Volume (V): Speaks by compatible sound volume to the situation
3 Voice (Voi): Avoids to use unsuitable voice tones (Boasting, whining, like a boss, sarcastic, etc.)
4 Facial expression (EF): Avoids the use of unsuitable facial expressions (Rude, grumpy, stuck-up view, etc.)
5 Posture (P): Uses appropppriate standing and sitting postures on the situation
6 Inter-Personal distance (PD): Uses appropppriate distance with others at standing or sitting.
7 Hygiene (H): Keeps body and clothes clean in good order
8 Body language (BL): Uses appropriate body language on the situation
9 Attitude (A): Uses Appropriate manners on the situation (Says “Please”, “Thank you”, “I’m sorry”, “Excuse me”).
10 Listening principles (LP): During listening, body language reflects “I’m listening to you” and “I think about the things said”
11 Stop/Change Topics (SCT): Continues to conversation or change the conversation subjects smoothly.
12 Speeches (S): Starts speech with salute, waits for order and listens during speech, and finishes with goodbye.
13 Interrupt/Cutting (IC): Interrupts appropppriately when needed

14 The rihght time and place (RTP): Thinks appropriate time and place for the things he will do and say

15 Official or Natural Formation (ONF): Knows why and how a more formal (befitting and respectful) or natural (relax and natural) behave.

All the steps of the study were planned and carried out according to the principles outlined in the Declaration of Helsinki (11). Local Ethics Committee Approval of Eskişehir Osmangazi University was also present for this study.

Statistical Analysis

SPSS (16.0) version was used for statistical Analysis. Chi-Square Test, Kruskal Wallis Variance Analysis, Mann Whitney U Test with Bonferroni Correction and Spearman's correlation rho efficient test were used.

$p<0.05$ was accepted as statistically significant.

Results

Demographic and personal characteristics of the students were shown on Table 1. In FM and FCS, percentage males were higher than females, whereas in FFA, bot genders were seen as equal number. In all faculties, most of the students were between 20-25 years old. In FM and FFA, the student were mainly the smallest children at their home, whereas in FCS, they are the biggest children at their home. The graduaded secondary schools were Science Secondary School and Anatolian Secondary School fort he students in FM; in FFA, the students were mainly graduaded from State State Secondary School, and in FCS, they were mainly graduaded from Anatolian Secondary School. Monthly income of the students were between 251-750 TR in FM and FFA, whereas in CM, the monthly income was higher than the others (501-1000 TR). In all faculties, students came from Metropolitans and cities. Regular sex activities were going on mostly in FFA (43%). In FCS (22%) and in FM (7%) of the students had regular sexual activities. Smoking was more observed in FFA (85%) and in FCS (43%). In FM, smoking was observed less compared to the other faculties (9%). Alcohol usage was present mostly in FFA (91%), and in FCS (83%). In FM, it was observed as 39%.

In all groups (Group 1-3), Body Esteem Scale and Social Communication Skills Rating Scale values were given as median (Table 2). For each of the items, the difference between group 1-3 was analyzed by Kruskal Wallis Variance Analysis. To find the values which caused the difference, pairwise comparisons were performed by Mann Whitney U Test with Bonferroni Correction (Table 3). The results showed that BES and SCSRS of the students of FFA were higher than the students of FM and FCS. As FM and FCS were compared, BES values were mainly higher in FCS and SCSRS values were mainly higher in the students of FM.

The correlation between SCSRS and Body Esteem Scale was analyzed by Spearman's correlation rho efficient test (Table 4, Figure 1)

-Eye contact scores were higher in students with higher BES scores of "lips", "Ears" and "appearance of eyes" and feet .

-Volume scores were higher in students with higher BES scores of body scent, nose, width of shoulders, figure or physique , appearance of stomach; and with lower BES scores of muscular strength, energy level and sex activities .

-Voice scores were higher in students with higher BES scores of reflexes, waist, thighs, chin, sex organs, physical condition and weight.

-Facial expression scores were higher in students with higher BES scores of physical stamina, waist, width of shoulders and health; and with lower BES scores of lips, biceps, cheeks/cheekbones face.

-Posture scores were higher in students with higher BES scores of physical stamina, reflexes, waist and energy level. Whereas Posture scores were lower in students with higher BES scores of muscular strength, biceps, physical coordination and arms.

-Personal distance scores were lower in students with higher BES scores of energy level and arms.

-Hygiene scores were higher in students with higher BES scores of cheeks/cheekbones and appearance of stomach. Whereas Hygiene scores were lower in students with higher BES scores of lips, arms, sex drive, sex activities and physical condition.

Table 1. Demographic and personal characteristics of the students

n		Faculty of Medicine (FM) (n=100)		Faculty of Fine Arts (FFA) (n=100)		Faculty of Communication Sciences (FCS) (n=100)		p*	X^2
		%	n	%	n	%			
Gender	Male	52	52,0	50	50,0	64	64,0	0,098	4,639
	Female	48	48,0	50	50,0	36	36,0		
Age	20-25	100	100,0	85	85,0	86	86,0	0,001	11,815
	26-30	0	0,0	13	13,0	9	9,0		
	31-35	0	0,0	2	2,0	5	5,0		
Which children in a family	The biggest	40	40,0	29	29,9	66	66,0	0,000	31,695
	Middle	14	14,0	26	26,8	10	10,0		
	The smallest	46	46,0	42	43,3	24	24,0		
Graduaded Secondary School	State Secondary School	3	3,0	51	51,0	43	43,0	0,000	56,232
	Anatolian Secondary School	42	42,0	23	23,0	52	52,0		
	Private College	12	12,0	0	0,0	4	4,0		
	Science Secondary School	43	43,0	8	8,0	1	1,0		
	Occupational Secondary School	0	0,0	18	18,0	0	0,0		
Monthly income	≤250 TR	7	7,0	15	15,0	13	13,0	0,000	19,369
	251-500 TR	39	39,0	25	25,0	8	8,0		
	501-750 TR	32	32,0	37	37,0	32	32,0		
	751-1000 TR	13	13,0	20	20,0	18	18,0		
	1001-1250 TR	3	3,0	0	0,0	8	8,0		
	≥1251 TR	4	4,0	3	3,0	21	21,0		
Live place before university	Metropolitan	46	46,0	50	50,0	75	75,0	0,038	4,286
	City	43	43,0	27	27,0	12	12,0		
	Province	8	8,0	23	23,0	7	7,0		
	Village	3	3,0	0	0,0	6	6,0		
Regular Sex	Yes	7	7,0	43	43,0	22	22,0	0,000	32,707
	No	81	81,0	57	57,0	74	74,0		
Bad Habits									
Smoking	Yes	9	9,0	85	85,0	43	43,0	0,000	131,920
	No	91	91,0	15	15,0	57	57,0		
Alcohol Usage	None	61	61,0	9	9,0	17	17,0	0,000	33,262
	Once a month	26	26,0	33	33,0	38	38,0		
	Once a week	10	10,0	18	18,0	20	20,0		
	A few times a week	3	3,0	27	27,0	25	25,0		
	Every day	0	0,0	13	13,0	0	0,0		

**p values shows the results of Chi-Square Test*

*Table 2. Body esteem scale and social communication skills rating scale results of the groups**

Body Esteem Scale					Social Communication Skills				
BES results (Median)	**FM**	**FFA**	**CF**	**P****	**SCSRS results (Median)**	**FM**	**FFA**	**CF**	**P****
Body Scent	4,0	5,0	4,0	0.005	Eye contact	3,0	3,0	3,0	0.000
Appetite	4,0	4,0	4,0	0.010	Volume	3,0	3,0	2,5	0.360
Nose	4,0	4,0	4,0	0.971	Voice	2,0	3,0	2,0	0.000
Physical stamina	4,0	4,0	3,5	0.023	Expression of face	2,0	3,0	2,0	0.000
Reflexes	4,0	5,0	4,0	0.001	Posture	2,0	3,0	2,0	0.000
Lips	4,0	4,0	5,0	0.000	Inter-personal distance	3,0	3,0	3,0	0.837
Muscular strength	4,0	4,0	4,0	0.108	Hygiene	3,0	3,0	3,0	0.001
Waist	4,0	4,0	4,0	0.008	Body language	3,0	3,0	3,0	0.002
Energy level	4,0	4,0	4,0	0.012	Attitude	3,0	3,0	3,0	0.151
Thighs	4,0	4,0	4,0	0.012	Listening principles	3,0	3,0	3,0	0.924
Ears	4,0	5,0	4,0	0.000	Stop/Change Topics	2,0	3,0	2,0	0.000
Biceps	4,0	4,0	4,0	0.048	Speeches	3,0	3,0	3,0	0.465
Chin	4,0	4,0	4,0	0.087	Intervention/Cutting	3,0	2,0	2,0	0.000
Body Build	4,0	4,0	4,0	0.005	The rihght time and place	3,0	3,0	2,0	0.013
Physical coordination	4,0	4,0	4,0	0.003	Official or Natural Formation	3,0	2,0	3,0	0.000
Agility	4,0	4,0	5,0	0.002					
Width of shoulders	4,0	4,0	4,0	0.251					
Arms	4,0	4,0	4,0	0.361					
Chest or breasts	4,0	4,0	4,0	0.345					
Appearance of eyes	4,0	5,0	4,0	0.000					
Cheeks/Cheekbones	4,0	4,0	4,5	0.039					
Hips	4,0	4,0	4,0	0.094					
Legs	4,0	4,0	4,0	0.007					
Figure or physique	4,0	4,0	4,0	0.014					
Sex drive	4,0	4,0	4,0	0.000					
Feet	3,0	4,0	4,0	0.000					
Sex organs	4,0	5,0	4,0	0.001					
Appearance of stomach	4,0	4,0	5,0	0.000					
Health	4,0	4,0	4,0	0.020					
Sex activities	4,0	4,0	4,0	0.037					
Body hair	4,0	4,0	4,0	0.105					
Physical condition	4,0	4,0	4,0	0.000					
Face	4,0	4,5	4,0	0.023					
Weight	4,0	4,0	5,0	0.000					

**FM: Faculty of Medicine, FFA: Faculty of Fine Arts, FCS: Faculty of Communication Sciences*
***p value shows the results of Kruskal Wallis Variance Analysis*

-Body language scores were higher in students with higher BES scores of chin and appearance of stomach. Whereas Body language scores were lower in students with higher BES scores of muscular strength, biceps, feet, sex activities and physical condition.

-Attitude scores were higher in students with higher BES scores of physical stamina, waist, ears, agility, legs, figure or physique, feet, appearance of stomach and face.

-Listening principles scores were higher in students with higher BES scores of appetite and appearance of stomach. Whereas Listening principles scores were lower in students with higher BES scores of body scent , energy level, body build and health

*Table 3. Pairwise comparisons by Mann Whitney U Test with Bonferroni Correection**

BES results	FM-FFA	FM-FCS	FFA-FCS	SCSRS results	FM-FFA	FM-FCS	FFA-FCS
	p	p	p		p	p	p
Body Scent	0.002	0.036	0.161	Eye contact	0.000	0.001	0.697
Appetite	0.002	0.138	0.152	Voice	0.000	0.688	0.000
Physical stamina	0.656	0.027	0.013	Expression of face	0.010	0.066	0.000
Reflexes	0.000	0.644	0.004	Posture	0.009	0.065	0.000
Lips	0.041	0.000	0.006	Hygiene	0.000	0.125	0.029
Waist	0.007	0.757	0.007	Body language	0.001	0.755	0.004
Energy level	0.028	0.460	0.005	Stop/Change Topics	0.037	0.000	0.000
Thighs	0.010	0.925	0.010	Intervention/Cutting	0.000	0.107	0.005
Ears	0.000	0.356	0.001	The rihght time and place	0.926	0.015	0.009
Biceps	0.119	0.354	0.016	Official or Natural Formation	0.000	0.940	0.000
Body Build	0.662	0.003	0.008				
Physical coordination	0.496	0.001	0.016				
Agility	0.034	0.001	0.114				
Appearance of eyes	0.000	0.835	0.001				
Legs	0.031	0.002	0.383				
Figure or physique	0.010	0.012	0.970				
Sex drive	0.000	0.004	0.293				
Feet	0.000	0.000	0.140				
Sex organs	0.000	0.164	0.025				
Appearance of stomach	0.004	0.000	0.040				
Health	0.383	0.049	0.008				
Sex activities	0.014	0.219	0.128				
Physical condition	0.000	0.329	0.000				
Face	0.013	0.904	0.023				
Weight	0.259	0.000	0.036				

**FM: Faculty of Medicine, FFA: Faculty of Fine Arts, FCS: Faculty of Communication Sciences*

-Stop/Change Topics scores were higher in students with higher BES scores of nose, physical stamina, waist, reflexes, energy level, thighs, ears, chin and appearance of stomach.Whereas Stop/Change Topics scores were lower in students with higher BES scores of muscular strength, biceps and arms.

- Speeches scores were higher in students with higher BES scores of lips, figure or physique and sex organs.

-Interrupt/Cutting scores were higher in students with higher BES scores of body scent and nose,

Whereas Interrupt/Cutting scores were lower in students with higher BES scores of muscular strength, energy level, physical coordination, agility, appearance of eyes, figure or physique, sex activities and physical condition.

-The right time and place scores were higher in students with higher BES scores of physical stamina, waist, width of shoulders, figure or physique, sex organs and physical condition;whereas The right time and place scores were lower in students with higher BES scores of Biceps.

-Official or Natural Formation scores were higher in students with higher BES scores of body build and appearance of stomach;whereas Official or Natural Formation scores were lower in students with higher BES scores of reflexes, ears, physical condition and face.

*Table 4. The relationship between social communication skills rating scale and body esteem scale**

Body Esteem Scale		Social Communication Skills Rating Scale														
		Eye contact	Volume	Voice	Expression of face	Posture	Inter-personal distance	Hygiene	Body language	Attitude	Listening principles	Stop/ Change Topics	Speeches	Intervention/ Cutting	The right time and place	Official or Natural Formation
Body Scent	r	0,020	0,137	0,055	-0,025	-0,031	-0,003	-0,018	0,109	-0,026	-0,116	0,003	-0,036	0,162	0,033	-0,076
	p	0,726	0,018	0,343	0,662	0,592	0,959	0,763	0,058	0,655	0,045	0,965	0,530	0,005	0,570	0,189
Appetite	r	0,098	0,029	-0,042	-0,061	-0,016	0,089	0,020	0,052	0,046	0,170	-0,021	-0,009	0,067	0,099	0,025
	p	0,090	0,617	0,465	0,291	0,787	0,124	0,724	0,371	0,427	0,003	0,715	0,871	0,248	0,085	0,662
Nose	r	0,092	0,115	0,006	-0,027	-0,030	0,006	0,021	0,093	0,016	0,113	0,121	0,006	0,135	0,087	0,061
	p	0,112	0,047	0,923	0,638	0,601	0,918	0,715	0,108	0,786	0,051	0,037	0,918	0,020	0,134	0,295
Physical stamina	r	0,058	-0,052	-0,013	0,156	0,177	-0,109	-0,003	0,069	0,137	-0,115	0,124	0,094	-0,083	0,122	0,022
	p	0,318	0,372	0,817	0,007	0,002	0,059	0,960	0,235	0,017	0,047	0,031	0,102	0,154	0,035	0,703
Reflexes	r	0,071	-0,037	0,121	0,026	0,127	0,048	-0,048	0,098	0,042	0,015	0,140	0,110	-0,056	0,066	-0,136
	p	0,219	0,526	0,036	0,656	0,028	0,409	0,407	0,091	0,471	0,801	0,015	0,058	0,336	0,254	0,018
Lips	r	0,157	0,039	0,043	-0,137	0,006	0,069	-0,160	0,088	0,119	0,063	0,054	0,208	0,038	0,053	-0,006
	p	0,006	0,500	0,454	0,017	0,919	0,231	0,005	0,127	0,040	0,277	0,351	0,000	0,510	0,357	0,915
Muscular strength	r	0,039	-0,167	-0,066	0,025	-0,135	-0,110	0,003	-0,187	-0,096	-0,104	-0,171	0,011	-0,116	-0,090	-0,031
	p	0,499	0,004	0,256	0,660	0,019	0,056	0,957	0,001	0,097	0,071	0,003	0,845	0,046	0,120	0,594
Waist	r	0,094	0,066	0,123	0,143	0,128	0,021	-0,001	0,043	0,176	-0,063	0,163	0,084	-0,002	0,187	-0,018
	p	0,105	0,253	0,033	0,013	0,027	0,713	0,991	0,455	0,002	0,275	0,005	0,146	0,970	0,001	0,754
Energy level	r	-0,023	-0,161	-0,029	0,018	0,115	-0,119	-0,083	-0,044	-0,020	-0,166	0,167	0,005	-0,248	0,018	-0,064
	p	0,693	0,005	0,619	0,752	0,047	0,040	0,152	0,452	0,731	0,004	0,004	0,935	0,000	0,752	0,266
Thighs	r	0,086	0,068	0,130	-0,035	-0,025	0,022	-0,106	0,095	0,084	0,006	0,125	0,109	-0,095	0,047	-0,099
	p	0,137	0,241	0,024	0,548	0,664	0,703	0,066	0,101	0,146	0,922	0,031	0,058	0,101	0,417	0,087
Ears	r	0,191	0,093	0,060	0,009	0,057	-0,034	-0,028	0,103	0,217	0,034	0,128	0,067	0,081	0,072	-0,167
	p	0,001	0,108	0,304	0,878	0,324	0,553	0,631	0,074	0,000	0,559	0,026	0,244	0,163	0,213	0,004
Biceps	r	-0,017	-0,063	-0,089	-0,173	-0,146	-0,082	0,019	-0,202	-0,015	-0,016	-0,142	0,004	0,036	-0,119	0,030
	p	0,765	0,279	0,126	0,003	0,011	0,156	0,749	0,000	0,791	0,785	0,014	0,940	0,535	0,039	0,600
Chin	r	0,031	0,103	0,118	0,014	0,005	0,044	-0,006	0,124	0,022	-0,005	0,166	0,056	-0,075	0,106	-0,110
	p	0,588	0,075	0,042	0,815	0,925	0,448	0,921	0,032	0,701	0,928	0,004	0,333	0,193	0,068	0,057
Body Build	r	-0,023	-0,026	-0,047	0,017	-0,108	-0,081	0,018	-0,062	-0,023	-0,191	-0,056	0,100	-0,041	-0,082	0,146
	p	0,690	0,655	0,419	0,771	0,061	0,161	0,761	0,286	0,685	0,001	0,333	0,084	0,484	0,158	0,011
Physical coordination	r	-0,020	-0,039	-0,062	-0,012	-0,116	-0,043	-0,048	-0,013	-0,024	-0,081	-0,071	0,032	-0,131	-0,039	0,060
	p	0,735	0,501	0,285	0,840	0,045	0,456	0,403	0,816	0,674	0,161	0,218	0,577	0,024	0,501	0,297
Agility	r	0,074	0,076	0,057	0,013	0,099	0,047	0,023	0,091	0,154	-0,053	-0,029	0,102	-0,155	0,035	0,025
	p	0,201	0,192	0,323	0,817	0,086	0,416	0,687	0,115	0,008	0,358	0,616	0,077	0,007	0,546	0,662

Width of shoulders	r	0,069	0,122	0,106	0,150	0,097	0,037	-0,056	0,069	0,103	-0,105	-0,027	-0,029	0,030	0,114	0,033
	p	0,233	0,035	0,067	0,009	0,094	0,525	0,332	0,231	0,076	0,070	0,646	0,616	0,601	0,049	0,573
Arms	r	0,008	-0,106	-0,087	0,090	-0,129	-0,143	-0,165	-0,029	0,101	0,110	-0,148	-0,032	0,023	-0,026	-0,012
	p	0,888	0,067	0,132	0,121	0,026	0,013	0,004	0,620	0,080	0,057	0,010	0,577	0,693	0,653	0,837
Chest or breasts	r	0,041	0,021	0,060	-0,084	-0,072	0,016	-0,086	0,039	0,083	0,052	-0,058	0,031	0,007	0,026	0,041
	p	0,482	0,717	0,303	0,146	0,212	0,786	0,139	0,506	0,150	0,368	0,319	0,592	0,897	0,660	0,476
Appearance of eyes	r	0,166	-0,044	0,082	0,025	0,080	-0,005	0,014	0,099	0,088	0,016	-0,017	0,092	-0,133	0,101	-0,033
	p	0,004	0,444	0,158	0,661	0,165	0,929	0,804	0,086	0,127	0,780	0,774	0,113	0,021	0,079	0,568
Cheeks/Cheekbones	r	0,094	-0,013	-0,065	-0,117	-0,087	-0,050	0,114	0,112	0,060	0,101	0,071	-0,054	0,005	-0,015	-0,018
	p	0,105	0,817	0,261	0,043	0,134	0,386	0,048	0,054	0,304	0,079	0,220	0,354	0,934	0,800	0,754
Hips	r	0,027	-0,025	0,010	-0,049	-0,111	-0,049	-0,032	-0,007	0,055	-0,014	0,011	0,045	0,027	0,006	-0,004
	p	0,641	0,670	0,859	0,402	0,056	0,395	0,579	0,906	0,342	0,812	0,847	0,433	0,643	0,921	0,942
Legs	r	0,023	-0,016	0,013	0,027	-0,056	-0,065	0,071	0,066	0,217	0,038	-0,095	0,071	-0,054	0,028	0,056
	p	0,690	0,777	0,823	0,638	0,331	0,262	0,221	0,255	0,000	0,515	0,099	0,220	0,348	0,629	0,331
Figure or physique	r	-0,002	0,137	0,110	-0,058	0,041	0,098	0,049	0,091	0,127	-0,059	0,063	0,127	-0,140	0,154	0,017
	p	0,976	0,017	0,057	0,313	0,480	0,091	0,396	0,116	0,028	0,306	0,280	0,028	0,016	0,007	0,771
Sex drive	r	0,102	0,008	0,045	-0,075	-0,008	-0,055	-0,121	0,067	0,063	-0,009	0,073	0,086	-0,039	0,060	-0,047
	p	0,079	0,893	0,438	0,195	0,893	0,341	0,037	0,251	0,281	0,874	0,208	0,139	0,507	0,303	0,423
Feet	r	0,184	0,061	0,036	0,018	0,074	0,000	-0,066	0,117	0,132	0,021	0,074	0,046	-0,068	0,072	-0,049
	p	0,001	0,294	0,534	0,754	0,202	0,994	0,251	0,044	0,022	0,716	0,200	0,428	0,243	0,213	0,399
Sex organs	r	0,085	0,003	0,223	-0,052	-0,045	0,070	-0,015	0,050	0,146	0,050	0,018	0,151	0,055	0,141	-0,106
	p	0,144	0,965	0,000	0,371	0,434	0,228	0,794	0,393	0,012	0,385	0,761	0,009	0,348	0,015	0,067
Appearance of stomach	r	0,095	0,157	0,021	0,048	0,060	0,093	0,176	0,196	0,231	0,132	0,117	0,057	0,093	0,080	0,123
	p	0,100	0,006	0,718	0,409	0,299	0,110	0,002	0,001	0,000	0,022	0,043	0,326	0,108	0,168	0,034
Health	r	-0,088	0,014	0,014	0,126	0,036	-0,099	-0,047	0,098	0,101	-0,174	0,102	0,008	-0,077	0,100	0,007
	p	0,128	0,804	0,803	0,029	0,536	0,087	0,421	0,092	0,082	0,003	0,078	0,895	0,185	0,083	0,901
Sex activities	r	-0,001	-0,156	-0,057	0,064	-0,100	-0,062	-0,130	-0,130	0,001	0,011	0,070	-0,058	-0,136	0,030	-0,028
	p	0,988	0,007	0,328	0,274	0,083	0,286	0,025	0,025	0,988	0,846	0,229	0,318	0,019	0,601	0,628
Body hair	r	0,087	-0,092	-0,114	0,058	0,032	-0,034	-0,031	-0,006	0,100	-0,095	-0,053	-0,089	-0,008	0,019	0,052
	p	0,155	0,128	0,060	0,340	0,597	0,574	0,610	0,923	0,100	0,117	0,388	0,144	0,902	0,751	0,395
Physical condition	r	0,020	-0,039	0,130	0,078	-0,027	0,063	-0,132	-0,145	0,092	-0,111	0,017	-0,045	-0,120	0,133	-0,144
	p	0,728	0,502	0,024	0,179	0,636	0,275	0,022	0,012	0,110	0,054	0,773	0,434	0,039	0,021	0,013
Face	r	0,044	-0,014	0,089	-0,113	-0,010	-0,025	0,006	-0,101	0,136	0,031	-0,099	0,071	-0,098	0,085	-0,159
	p	0,451	0,810	0,124	0,050	0,861	0,668	0,921	0,079	0,018	0,598	0,086	0,222	0,090	0,143	0,006
Weight	r	0,079	-0,004	-0,114	0,021	-0,058	-0,015	0,090	0,007	0,101	-0,015	-0,089	0,000	0,079	0,037	0,019
	p	0,175	0,941	0,049	0,719	0,317	0,790	0,120	0,900	0,080	0,802	0,124	0,995	0,174	0,525	0,745

**p value shows the results of Spearman's correlation rho efficient test*

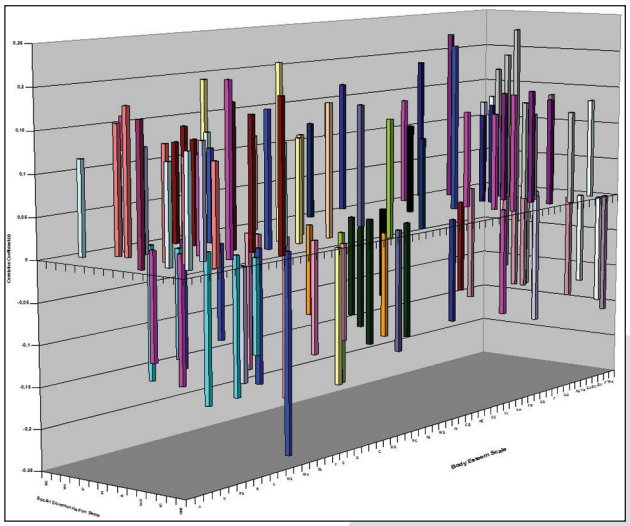

Figure 1. Correlation coefficients between Social Communication Skills and Body Esteem Scale in all groups

Discussion

The basis on which people make social judgments from the image of a face remains an important open problem in fields ranging from psychology to neuroscience and economics. Multiple cues from facial appearance influence the judgments that viewers make. It was reported that a novel facial cue influencing a range of social judgments as a function of interpersonal distance, an effect that may be processed implicitly (12).

Social cognition is the ability to analyze, sum up, and establish goals for a situation, then decide what strategies would be appropriate to achieve those goals. This includes judging how to talk to people in different situations (e.g., it is inappropriate to send a singing telegram announcing that someone has passed away). Social cognition also involves gauging whose turn it is to talk in a conversation based on facial expressions and other nonverbal cues of conversational partners (6).

In the present study, we investigated the relationship between Social Communication skills and Body Esteem Scales (BES) of the university students. They were living Metropolitans and cities mainly. Monthly incomes of Faculty of Communication Sciences (FCS) students were higher than the students of Faculty of Medicine (FM) and Faculty of Fine Arts (FFA). It may be concluded as the children of families with higher socio-economical level preferred Faculty of Communication Sciences. Regular sex activities were going on mostly in FFA (43%). In FCS (22%) and in FM (7%) of the students had regular sexual activities. Smoking was more observed in FFA (85%) and in FCS (43%). In FM, smoking was observed less compared to the other faculties (9%). Alcohol usage was present mostly in FFA (91%), and in FCS (83%). In FM, it was observed as 39%.

Our results showed that BES and SCSRS of the students of FFA were higher than the students of FM and FCS. As FM and FCS were compared, BES values were mainly higher in FCS and SCSRS values were mainly higher in the students of FM.

The relationship between SCSRS and Body Esteem Scale was analyzed by correlation test. Our results showed that the students who were happy with their lips, ears, width of shoulders, figure or physique , appearance of stomach, generally had higher social communication skill scores. In Turkish population, facial beauty is very important for most of the subjects; and it may cause to greater self-confidence.

Whereas higher muscular strength, agility, higher energy levels or higher sex activities were related to lower social communication results. In Turkey, active sexual life before marriage is not accepted as a cultural norms. Because of that, the students with regular sex and active sexual life may have feelings of guilt; and this may be reflected to their social relations and communications by negative ways. Surprisingly, hygiene scores were also lower in students with higher BES scores of sex drive and sex activities. This results should notice the educational planners for giving detailed information for hygiene and safer sexual activities.

For body language scores, BES scores of chin and appearance of stomach were found as statistically significant. The students having good appearence, may have more self-confidence; and these subjects may use body language appropppriately.

Personal distance begins about an arm's length away; starting around 18 inches (46 cm) from the person and ending about 4 feet (122 cm) away. This space is used in conversations with friends, to chat with associates, and in group discussions. Social distance ranges from 4 to 8 feet (1.2 m - 2.4 m) away from the person and is reserved for strangers, newly formed groups, and new acquaintances. Public distance includes anything more than 8 feet (2.4 m)

away, and is used for speeches, lectures, and theater. Public distance is essentially that range reserved for larger audiences (2,13). In the present study, it was found that personal distance scores were lower in students with higher BES scores of energy level and arms. We conclude that the students with higher energy levels may be more active and moveable; and these students may have not give enough attention to maintain interpersonal distance.

On attitudes and communication skills in the community, their perceptions of bodies have an important role. While the chilgren being growing up, they should be trained appropppriately and should be given indoctrination on the communication skills and behaviors in the society. Not only the visual aspects, but also the mental state of people, ideas, and behaviors are important; and these issues should be told the children from childhood.

We concluded that Social Communication skills were affected by Body Esteem Scales of the students. At the same time, faculty preferences influence body perception, and social communication skills. The students of Fine Arts Faculty had higher BES and SCSRS values compare to the other two faculties. From childhood and during upbringing, social norms, view of sexuality, cultural structure of society for the perception of the shape and beauty influence the perception of the body, and this changes their social communication skills.

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Evaluation of levels Adiponectin, insulin-like growth factor-1, and insulin-like growth factor binding protein-3 in patients with acute myeloid leukemia and acute lymphoid leukemia

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Abstract

Objective: Adiponectin is an apoptosis-inducing protein secreted by adipocytes. In many different types of cancer, insulin-like growth factor-1 (IGF-1) is an important growth and antiapopitotic factor for cancer cells. Insulin-like growth factor binding protein-3 (IGFBP-3) induces apoptosis and inhibits cell growth without interaction with IGF-1. The aim of our study was to compare serum levels of adiponectin, IGF-1, and IGFBP-3 of acute leukemia patients to a healthy control group.

Materials and Methods: We sampled 8-10 mL venous blood from 15 ALL patients aged 18-62 years, 15 AML patients aged 17-67 years, and 15 healthy volunteers aged 18-62 years. All diagnoses were made in the Hematology and Oncology Department of Ataturk University Medical Faculty. The samples were centrifuged and then serums kept at -80°C until tested. Serum adiponectin levels were measured with a ready-to-use ELISA kit (Ray Bio Human Adiponectin ELISA Kit Protocol ELH-Adiponectin-001). Serum IGF-1 and IGFBP-3 levels were measured with immunofluorescence Assay (IFA) and immunoradiometric Assay (IRMA) methods.

Results: Average serum levels of IGF-1 of ALL and AML groups (52.69+-89.1 and 133.81+-69.4 ng/mL, respectively) were lower than the control group (170.89+-79.1 ng/mL), but the difference was not statistically significant ($p>0.05$). IGFBP-3 average serum levels of ALL and AML groups (3.45+-1.4 and 2.93+-1.4 ng/mL) were lower than the control group (4.66+-0.8 ng/mL), and the difference was statistically significant ($p<0.001$). Adiponectin average serum levels of both AML and ALL groups (1649+-805 and 1649+-1529 pg/mL) were higher than the control group (1539+-1255 pg/mL), but the difference was not statistically significant ($p>0.05$).

Conclusion: Although both IGF-1 and IGFBP-3 serum levels of ALL and AML groups were lower compared to the control group, the only the difference of the IGFBP-3 levels was statistically significant. In our study, we speculated that low IGFBP-3 levels may be regarded as a risk factor for acute leukemia, as in other cancer types.

Key words: ALL, AML, IGF–I, IGFBP–3, Adiponectin.

Introduction

Acute leukemia's are neoplastic diseases of the hematopoietic system that result from the clonal proliferation of immature cells. Ultimately, hematopoietic cell maturation pauses and an aberrant clonal transformation occurs. As a result, the so-called blast;immature myeloid or lymphoid cells quickly accumulate and gradually replace the bone marrow. Loss of normal bone marrow function leads to common leukemia complications. Leukemia constitutes approximately 3% of all cancers. The etiologic cause of acute leukemia is not yet precisely known (1,2).

Adiponectin is a specific secretory adipocyte protein that induces apoptosis (3). Adiponectin is an important negative regulatory protein on the immune and hematopoietic systems; this regulatory effect is provided via the suppression of granulocyte macrophage colony stimulating factor (GM-CSF). It has been recently discovered that it inhibits the proliferation of myeloid series without affecting the lymphoid cell lines (4.5).

Insulin-like growth factor I (IGF-I), is an important growth and antiapoptotic factor for cancer cells in many types of cancers. Insulin-like growth factor binding protein 3 (IGFBP-3) stimulates apoptosis and inhibits growth independent from IGF-I. Recently, many epidemiological studies have reported that low serum IGF-I and high IGFBP-3 levels or an elevated rate of IGF-I/IGFBP-3 levels are associated with an increased risk for many cancer types, including breast, prostate, colon, and lung cancers (6). In addition to these, recently it has been claimed that IGFBP-3 and IGF-1 can be used as a marker at diagnosis and the evaluation of therapy in childhood ALL (7).

In the light the above data, we aimed to investigate the serum levels of adiponectin, IGF-I, and IGFBP-3 in acute myeloid and lymphoid leukemia (AML and ALL) patients and compare them with healthy subjects. We also aimed to investigate the relationship of serum levels of adiponectin, IGF-I, and IGFBP-3 with AML and ALL.

Materials and Methods

A total of 30 volunteer patients who were diagnosed either AML or ALL in the Hematology Department of Internal Medicine of Ataturk University Faculty of Medicine were enrolled in this study. Patients with a known history of other systemic disease were excluded. Fifteen healthy volunteer subjects who had no problems according to the results of medical and laboratory examination results served as controls.

Nine ALL patients were female and six were male. The age distribution of ALL patients was 18-62 years. Ten AML patients were male and five were female. The age distribution of AML patients was 17-67 years. Eight participants from the healthy subject control group were female and seven were male.

A blood sample of approximately 10 ml was collected via a 10 ml disposable plastic syringe from the cubital vein of all the patients and healthy subjects. A 5 ml of blood sample was centrifuged at 4200 rpm for 5 minutes. Centrifuged samples were stored in a deep freezer at -80° C to investigate the serum levels of adiponectin. A resting 5 ml of blood sample was analyzed in the hormone division of Atatürk University's Biochemistry Laboratory for IGF-I and IGFBL-3 levels immediately after sampling. After all the samples were collected, stored samples for serum adiponectin levels were disintegrated by placing them in a +4° C fridge for 12 hours. Sera were brought to room temperature and the tests were performed.

Serum levels of IGF-I and IGFBP-3 were analyzed at the hormone laboratory using Biosource® brand commercial kits. IGF-I was analyzed via immunofluorescent assay (IFA) and IGFBP-3 was analyzed via immunoradiometric assay (IRMA) methods. A Sampler System® brand adjustable automatic pipette was used during the analysis. Counts were made with a Gamma C-12® brand counter. The serum level of adiponectin was analyzed via a commercially available RayBio® Human Adiponectin ELISA Kit (Protocol-ELH-001).

Statistical analysis

Statistical analyses were performed using the SPSS 11.5 program. Data was given as numbers, percentage, mean, and standard deviation. An ANOVA test was used to compare categorical variables between groups and Ducan test was used for correlations of categorical variables. A p value of <0.05 was considered statistically significant.

Results

The mean serum level of IGF-I in patients with ALL was 152.69 ± 89.1 ng/ml. However, the mean serum level of IGF-I in healthy subjects was measured as 170.89 ± 79.1 ng/ml. The serum IGF-I levels of ALL patients was detected to be lower than the control group, though this difference was not statistically significant ($p> 0.05$). The mean serum IGF- I level of AML patients and the control group was 133.81 ± 69.4 ng/ml and 170.89 ± 79.1 ng/ml, respectively. IGF-I levels in patients with AML were lower than the control group, although this difference was no statistically significant ($p> 0.05$). IGF-I values in patients with ALL was higher than AML patients, although this difference was not statistically significant ($p> 0.05$).

The mean serum level of IGFBP-3 of ALL patients and control group was detected as 3.45±1.4 ng/ml and 4.66±0.8 ng/ml, respectively. The mean serum IGFBP-3 level of ALL patients was statistically significantly lower than the control group ($p<0.001$). The mean serum IGFBP-3 level of

AML patients was detected as 2,93±1.4 ng/ml. The mean serum level of AML patients was detected to be statistically significantly lower than the control group ($p<0.001$). There was no statistically significant difference in serum levels in the IGFBP-3 levels of AML and ALL patients ($p>0.05$).

The measured average value of serum adiponectin level in patients with ALL and the control group were 1649 ± 805 pg/ml and 1539.33 ± 1255 pg/ml in respectively. The average value of adiponectin in the control group was lower than the average value of adiponectin in patients with ALL. This difference was not statistically significant ($p> 0.05$). The measured average value of the serum level of adiponectin in patients with AML was 1648.86 ± 1529 pg/ml. Although the mean serum level of adiponectin of AML patients was higher than the control group, this difference was not statistically significant ($p> 0.05$). The average value of serum adiponectin levels in patients with ALL was higher than in patients with AML. This difference was not statistically significant ($p> 0.05$).

Discussion

Several epidemiological studies have reported that low serum IGF-I and high IGFBP-3 levels or an elevated rate of IGF-I/IGFBP-3 is associated with an increased risk for many cancer types, including breast, prostate, colon, and lung cancers. It was reported that interventions to antagonize IGF-I receptor (IGF-IR) stimulation or to increase IGFBP-3 function can cease tumor cell growth in models of human cancer. It was also detected that serum IGF-I levels increased and IGFBP-3 levels decreased in many tumor types (8.9).

Petridou et al. (10) investigated the presence of any correlation between IGF-I, IGF-II, and survival, and found no correlation. It was speculated that IGFBP-3 reduces mortality. Jing Ma et al. (11) found an increased risk of colorectal cancer in patients with high serum IGF-I levels. High serum levels of IGFBP-3 have been reported to reduce the risk of colorectal cancer. Chan et al. (12) found that the risk of prostate cancer in men with high IGF-I levels is significantly higher compared to individuals with lower levels of IGF-I. Wu et al. (13) reported that serum IGF-I levels in patients with lung cancer was higher than the control group. They detected that serum IGFBP-3 levels are also lower in patients with lung cancer compared to the control group. In our study, although serum IGF-I levels of patients with ALL and AML were found to be lower than in the control group, this difference was not statistically significant. Our results were not correlated with the results of Petridou, Jing Ma, Chan, and Wu et al. This miscorrelation may be a result of the fact that our study group was small in number, the investigated cancer types of the studies were different than our study, or the patient groups had different eating habits. In this study, we detected that the level of IGFBP-3 in patients with AML and with ALL were lower than the control group, which correlated with the literature. Despite the fact that the measured IGFBP-3 levels in adult patients with AML were lower than adult patients with ALL, the difference was not statistically significant.

Adipose tissue, which is considered biologically active, secretes many molecules regarded as inflammatory markers that believed to be involved in obesity dysregulation, development of insulin resistance, and vascular diseases (14). Iversen et al. (5) demonstrated that adiponectin obtained from adipocytes inhibits the human myelomonocytic precursor cells and B-lymphopoiesis of rats in their study. In this study investigators detected that cytokines inhibit short and long term growth of immature CD34 positive bone marrow cells of patients with AML in cell cultures. It was speculated that the inhibition of hematopoiesis partially resulted from TNF-alpha and adiponectin because an increase was detected in both of the cytokine levels in the bone marrow of AML patients. TNF-α production and mRNA expression from immature blast cells of AML patients, as well as mRNA expression from primary human osteoblasts were detected; on the other hand, such an expression and secretion was not detected in the immature hematopoietic cells of healthy subjects. A statistically significant difference was not detected in either of the cytokine serum levels between the patient and control groups. Serum levels of TNF-α and adiponectin decreased in seven patients who had complete remission. Petridou et al. (3) investigated serum adiponectin levels of childhood AML patients with a control group. They reported that the serum adiponectin level of AML patients was lower than the control group. However, they did not de-

tect a significant difference for serum adiponectin of patients with T or B ALL and the control group. Iversen et al. (5) demonstrated that blast cells from the bone marrow of patients with AML had mRNA expression for the production of adiponectin, but this condition was not true for the cells in normal individuals. On the other hand, Petridou et al. reported (3) serum adiponectin levels of the children with AML were lower than the control group They are not a significant relationship between patients with ALL have been reported.

The present *study* has certain *limitations*. This miscorrelation may results from reasons such as: our study group was small in number, the investigated cancer types of the studies were different than our study, or that the patient groups had different eating habits.

Although our study has limitations, this study is a unique contribution to the literature that investigates adiponectin, IGFBP-3, and IGF-1 levels in adult ALL and AML patients. According to our study, serum IGFBP-3 levels of ALL and AML patients are significantly lower than the healthy control group; thus, it may be a risk factor for ALL and AML. Furthermore, high adiponectin levels of AML and ALL patients may be a predisposing-condition for these diseases. In order to strengthen these speculations, further studies with larger patient groups are necessary.

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Estimation of insular tumor size using tracing method

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Abstract

Insula is the most significant area for taste, the control of motor speech, vestibular functions and the cardiovascular sympathetic control. Although there are anatomical and clinical studies related to insula in the literature, there is restricted data for surgical approach of the area. We aimed to measure the area of insular space occupied the lesion on MRI sections using manual tracing technique. Additionally, we planned on a clinical and microscopic evaluation of our case with insular tumor in order to remove the tumor with safety surgical procedure.

The area of the tumor and size as mm^2 were measured by the tracing method on sagittal and axial MRI slices. In the presented patient with Glioblastoma Multiforme (GBM) of the insular region emphasizing imaging and light microscopic findings and the surgical experience were displayed.

We measured 30x20x25 mm sized lesion on MRI slices in the left insula. The top of the tumor lay toward the lateral sulcus. The maximum size of the tumor was measured as 154 mm^2. The patient underwent a left pterional craniotomy by performing tangential dissection of hairy skin, subcutaneous fat tissue with a Sylvian approach in order to excise the mass. Meticulous micro dissections of insular region were carried out and then the mass was identified clearly. Routine microscopic evaluation of the mass revealed a lesion composed of highly anaplastic glial cells with a prominent proportion of tumor cells characterized by classic round cytoplasmic inclusions and eccentrically positioned nuclei, micro vascular proliferation and areas of necrosis, defining the diagnosis of GBM. Postoperatively there was no neurodeficit, epileptic seizure or the other symptoms, and the patient was discharged at 7th day.

This study provides a safety surgical zone for surgeons about insular region to reach the deep area of the temporal lobe, to protect the major anatomical structures and to avoid intra operative complications.

Key words: Insula, Insular tumor, Neurosurgery, MRI, Manual tracing.

Introduction

The insula of Reil is located in the depth of the brain. It is named after the German anatomist, physiologist and psychiatrist, Johann Christian Reil (1759-1813), who in 1809 first described this anatomically and functionally complex structure. The insula is one of the paralimbic structures and constitutes the invaginated portion of the cerebral cortex, forming the base of the Sylvian fissure [7,18,21].

The human insula is associated with olfactory and autonomic functions, area taste function and also with the somatic sensory, auditory and vision functions [11,12,16]. Although the morphology and the function of the central nervous system have been researched for long, there are still some important aspects which keep their secrets. Current literature lacks a precise description of the cortical anatomy of the insula, perhaps because classical morphomethric methods, including imaging techniques, struggle to reach this deep structure, covered by its rich vascular network. Knowledge obtained from the stimulation of cerebral cortex intraoperatively and the clinical findings of the patients with insular lesions contribute to the understanding of the human insula [2, 8-10,13,14,16].

In the present study, to determine the borders and the size of the tumor, tracing technique was used to provide safety neurosurgical procedure. We presented a patient with Glioblastoma Multiforme (GBM) of the insular region, emphasizing the imaging findings and the review of the literature reported case. The present study evaluated anatomic features of GBM and relates adjacent structures and outcomes with the use of the surgical treatment approach for the patient with the

tumor. The mass was also measured by tracing method using Osiris 4.19 software programme. Evaluation of the method was of importance during surgical planning process.

Material and methods

Case analysis

A 65-year-old right handed woman applied to Kocatepe University Faculty of Medicine, Department of Neurosurgery Clinic with a three-month history of progressive headache, drowsiness, dizzartry, nausea, dizziness and epileptic seizures. Physical examination revealed nuchal rigidity, decrease in muscle strength in the right extremities and Babinski sign positive in the right. The MRI examination showed an irregular rounded hyper intense, 30x20x25mm sized lesion at the left insular region in both T1 and T2. After contrast administration the mass showed heterogeneous enhancement (Figure 1). With these findings, the case was operated by neurosurgeons.

The patient underwent a left pterional craniotomy by performing tangential dissection of hairy skin, subcutaneous fat tissue with a Sylvian approach in order to excise the mass. Meticulous micro dissections of insular region were carried out and then the mass was identified clearly. During the surgery a yellow-gray swelling and bleeding mass was encountered and it was gross totally excised and confirmed histopathologically.

Morphometric Analysis

Axial MRI slice of the case was analyzed by semi-automated procedure, which validated by means of comparison with manual tracing technique. MR imaging was used for size of the insular lesion by using 1.5 Tesla Picker Instrument. On coronal plane, SE T1A (TR: 500-700 ms; TE: 10-30 ms) cross-section was made and T1 weighted coronal image was recorded. The section was magnified 3-4 times by way of Osiris 4.19 software programme using tracing method on surrounding of the tumor and then obtaining numeric data of the maximum lesion area as mm^2 in insula.

Results

We measured 30x20x25 mm sized lesion on MRI slices in the left insula (Figure 1). The top of the tumor lay toward the lateral sulcus. The maximum size of the tumor was measured as 154 mm^2 using tracing method on MRI coronal slice (Figure 2). Routine microscopic evaluation revealed a lesion composed of highly anaplastic glial cells with a prominent proportion of tumor cells characterized by classic round cytoplasmic inclusions and eccentrically positioned nuclei, micro vascular proliferation and areas of necrosis, defining the diagnosis of GBM (Figure 3).

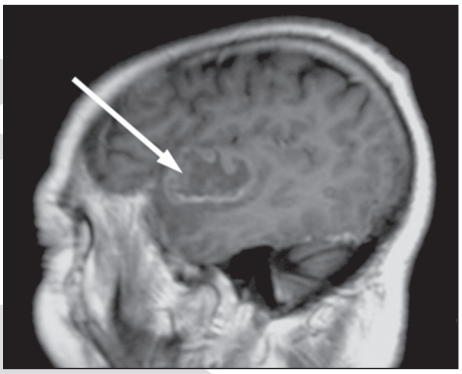

Figure 1. Lesion at the left insular region on the sagittal MRI

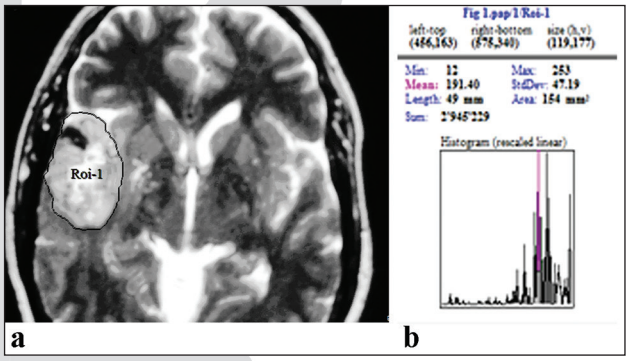

Figure 2. (a) The tumor was showed by using tracing method on MRI coronal slice
(b) The coronal section was magnified 3-4 times by way of Osiris 4.19 software programme

3x100mg/day diphenylhidantoin was used as antiepileptic drug therapy after postoperative early term. Postoperatively there was no neurodeficit. The patient was discharged at 7th day. At this time radiotherapy was recommended, but the patient refused the treatment. Epileptic seizure or the other symptoms, was not encountered following the first 6 months.

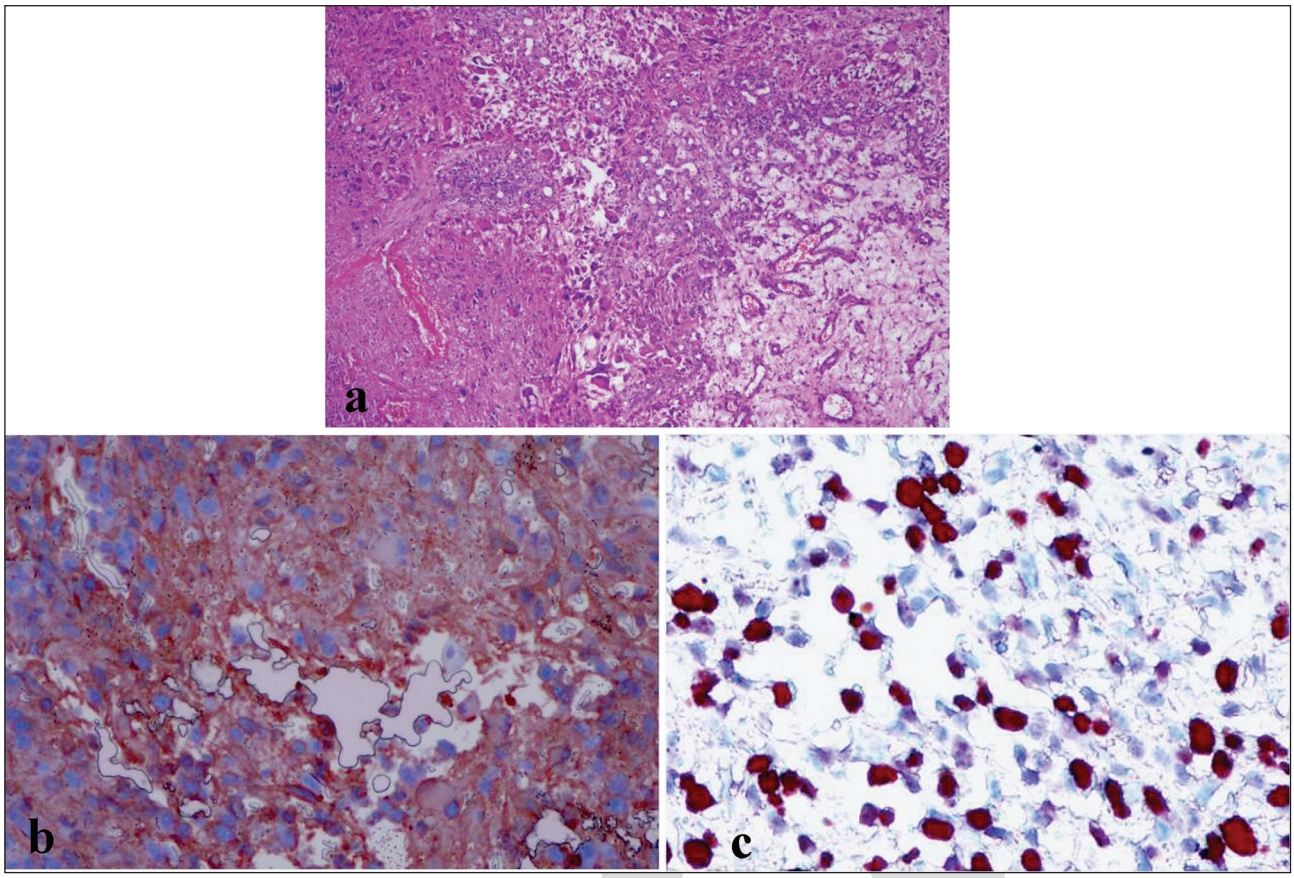

Figure 3. (a) Necrosis on left side and pleomorphic atypic glia cells of glioblastoma which contains vascular endothelial proliferations on right side (H&E,X40)
(b) Anti-GFAP (+) immunohystochemical staining of tumoral cells (GFAP, X200)
(c) The proliferation index as 25% with immunohystochemical staining of the tumoral cells by Ki67 anticore. (Ki67, X200)

Discussion

Insula is the most significant area for taste, the control of motor speech, vestibular functions and the cardiovascular sympathetic control [15,17]. Native-space quantification of the insula may be valuable in the study of neurodevelopmental or neuropsychiatric disorders related to anxiety and social behavior [4]. The definition of cortical organization of the brain has recorded fairly important stages since the beginning of 1900s. In his compilation on insula functions Augustine reported [2] that Oskar and Vogt were the pioneers with the studies about the cortical constructing they performed. Korbinian Brodmann proposed the most valid map today [2]. In the literature, insula views are seen firstly in the drawings Vesalius achieved on the axial plane. Approximately 50 years after insula was characterized, lesions in aphasia patients were defined to be in the caudal part of gyrus frontalis inferior (Broca area) by Broca. However, one of the patients did not have the lesion in this area; the lesion was detected in insula and nearby cortical area. Insular tumor surgery carries substantial complication rates. Nevertheless, there are few and limited studies about importance of the insular anatomy in neurosurgical operations. Prevention of the vascular structures or understanding of anatomic characteristics of adjacent insular region are important for postoperative survive of neurosurgical operations for the cases with insular tumors. So, we also summarized our clinical experience with microsurgical resection of the cases with insular tumor.

GBMs are highly malignant tumors characterized by microvascular proliferation and the pseudopalisading pattern of necrosis [6]. Patients with glioblastomas and/or age over 60 years like our case require a more cautious approach [19]. In the studies by Varnavas et al [22] and Ture et al [20] the resection of insular tumors existing especially

in dominant hemisphere was emphasized to be risky and difficult due to the complex anatomy of insula. In the present case, we thought that disarthria symptom was related to compression of the adjacent structures because of mass effect and edema nearby Broca area in dominant hemisphere. In a study by Bouilleret et al [3] neuroanatomical correlation of the initial symptoms of the epileptic seizures was detected by positron emission tomography (PET). Due to the connections of insula with olfactory area, corpus amygdaloideum, entorhinal cortex, gyrus cinguli and hippocampus (temporolimbic structures), it was considered that it was involved in the limbic epilepsy etiology. And also, it was determined that there was a similarity between the symptoms of the seizures of the patients with temporal lobe epilepsy and the symptoms occurring as a result of the insular cortex stimulation. This similarity may be explained thanks to the massive connections existing between lobus insularis and temporo-lymbic structures [5]. In the present case after tumor resection, epileptic seizures were controlled by an antiepileptic drug to avoid possible epileptic seizures after surgery.

Further investigations may document the role of the insula in the pathology of partial epilepsy [1]. In their researches related to insula tumors Zentner et al [23] and Lang et al [5] emphasized that the differentiation of sulcus preinsularis from the surrounding tissue was significant in identifying the tumor resection plans by mentioning sulcus lateralis in order to avoid the surgical and postoperative clinical complications. It is announced that vascular structures and capsula interna carry the damage risk in insular or insular-opercular tumors in dominant hemispheres. The tracing method is found to be a reliable, simple, computed, manual and efficient method for estimating exact location and maximum diameter of the occupant lesion on MRI slice. We may use that numeric data of the case with insular region tumor to prevent chirurgical injuries of surrounding intact brain tissue during surgical intervention.

Conclusion

We suggest that the results of our study may be beneficial for the neurosurgeons. The stereological measure of the insular tumor used in this study, will help surgeons to extract the masses in a safer way and also to protect the surrounding tissue and vasculature during surgical approach.

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A pin in the uterus: The dilemma of migration path

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Abstract

A 19-year old girl with dysmenorrhea, foul smelling vaginal discharge and chills/tremor episodes presented to our hospital. Her examination showed that she was virgin and she had suprapubic tenderness and fever (39 ºC). Pelvic ultrasound revealed a highly echogenic linear structure within the uterus that was 3-4 cm in size. Abdominal computerized tomography scan suggested the presence of a pin-like foreign body in the uterus. Because she did not accept the hysteroscopic approach, mini-laparotomy was used to enter the abdomen. The pinhead was localized in the fundus under fluoroscopic guidance and the pin was reached and removed through an uterine incision. It was unclear from her medical history that how the pin reached the uterus. In a virgin patient, chronic dysmenorrhea, foul smelling vaginal discharge and endometritis suggest the presence of a foreign body.

Key words: Foreign body, Uterus, Pin.

Introduction

In an adolescent girl, long-lasting foul smelling vaginal discharge can be attributed to vaginitis, endometritis or pelvic inflammatory disease, vulvar skin diseases and, rarely, neoplasia or presence of intravaginal/intrauterine foreign body. While the presence of an intravaginal foreign body is typically detected during childhood, it is more commonly due to masturbation or mental or psychiatric disorders among adolescents; occasionally, it may also indicate sexual abuse (1). Several case reports in the literature confirm that intrauterine foreign bodies are often remnants of intrauterine devices or pregnancy products (2). Our report aims to present a novel finding of an intrauterine pin in a 19-year old virgin and discuss its potential migration path.

Case report

A 19-year old unmarried girl with dysmenorrhea that lasted for 6 months and foul smelling vaginal discharge for the previous 2 months was presented to our hospital with the complaint of episodes of chills and tremors within the previous 2 days. The patient's blood pressure was normal, pulse was tachycardic, and her body temperature was 39 °C. Pelvic ultrasound showed a hyperechogenic body standing on the fundus with a size of approximately 3-4 cm, the majority of which lied in the uterine cavity, suggesting the presence of an intrauterine device (Figure 1). It was unclear from her medical history that how the pin reached this uterine location.

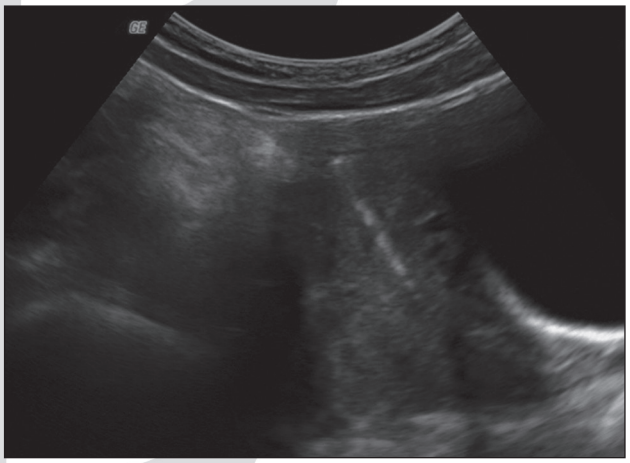

Figure 1. Trans-abdominal ultrasound shows the hyperecogenic foreign body in the uterine cavity

In the examination, hymen was intact with a egg-shaped hole in the center and yellow-green foul smelling vaginal discharge was detected. Physical examination did not reveal any finding of sexual abuse. Vaginal discharge was sampled for the culture. Her white cell count was 14800/L and 1-hour sedimentation was 52 mm/hour. Abdominal examination revealed a tenderness upon palpation. Computerized tomography was completed for differential diagnosis and determined that the

foreign body in the uterus could be a pin (Figure 2). Cultures of vaginal swabs demonstrated the presence of *Staphylococcus epidermidis*.

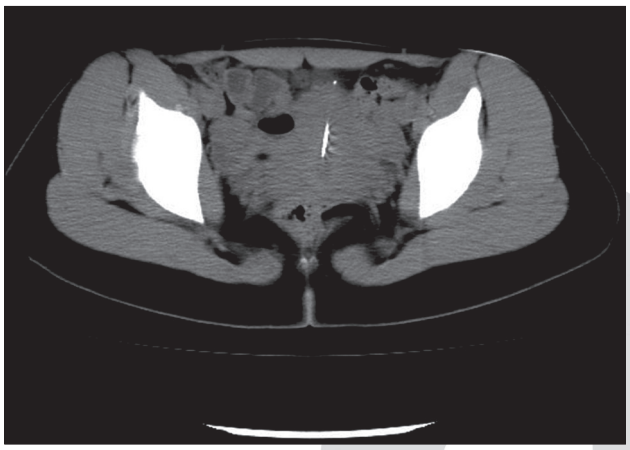

Figure 2. CT scan reveals the location of the foreign body. An axial section of the patient's pelvic scan. Note the hyper-intense pinhead above the uterus and the hyper-intense pinhead in the uterus

Initially, hysteroscopic intervention was recommended and antibiotics were given for the pelvic infection prior to the intervention (Cefotetan 2x1 g plus Doxycycline 100 mg 2x1). Based on personal beliefs, the patient refused a vaginal intervention approach. Upon the consideration of the need for hysteretomy in order to remove the foreign body, we entered into the abdomen using mini-laparotomy. The pinhead was embedded in the myometrium and was removed from the cavity (Figure 3).

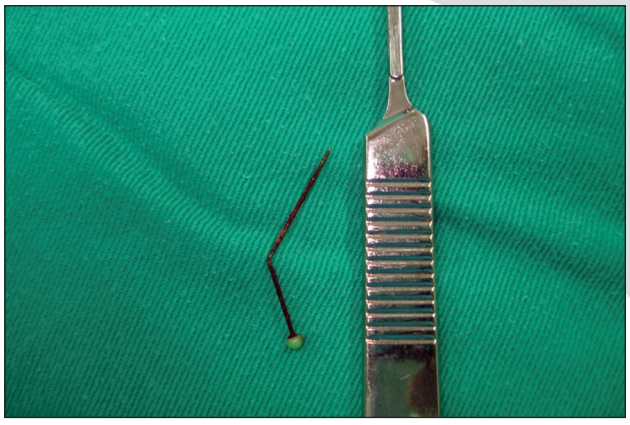

Figure 3. Foreign body (pin)

The patient had parenteral antibiotic therapy for an additional two days and did not experience fever or pain. The patient and her family were consulted by the department of psychiatry and no psychiatric pathology was encountered. She was discharged three days after the operation with oral antibiotic therapy (Doxycycline 100 mg 2x1). Two weeks after the operation, no pathological findings were found.

Discussion

The width of the external vaginal orifice is approximately 1 cm in an adolescent girl; therefore, it is not difficult to consider the insertion of a foreign body into the vagina through this route. However, unless it is placed due to sexual abuse or perversion, it is unlikely that a foreign body could move through the vagina, pass through the nulliparous cervical canal and reach the uterus.

Alternatively, given reported cases in which a pin is held in the mouth for subsequent use to fix a head scarf is swallowed or aspirated and passes to the respiratory or gastrointestinal system (3,4), we hypothesized that the pin migrated to the genitourinary system through these systems. This hypothesis was also supported by the fact that the pinhead was embedded in the myometrium of the uterine fundus. However, in this case, the patient would be expected to have signs and symptoms from the respiratory or gastrointestinal tracts, such as abdominal pain, cough, bleeding, and perforation. Given that, among sharp bodies that are swallowed, 93% are symptomatic and 70% result in perforation. Migration of a foreign body to the uterus after being swallowed is not likely (3).

Because the patient was virgin, had no history of sexual intercourse or sexual abuse, and had no mental or psychiatric disorders, it seems less likely that the pin entered the uterus by the ascendant path. However, given that the patient's history did not contain any complaint concerning the gastrointestinal or respiratory systems and that operative examination did not reveal evidence in the abdomen for the migration of this foreign body, the theory of descendent migration becomes less possible.

Although the therapeutic approach remains the same regardless of the route by which the foreign body reached the uterus, the question regarding the specific route is undoubtedly interesting for the clinician. Furthermore, it should be kept in mind that the ascendant migration path is instruc-

tive in terms of sexual abuse or, if a descendent migration is suspected, masked problems may be elucidated by adequately assessing the respiratory and gastrointestinal systems.

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A case of Dandy-Walker Syndrome associated with small facial hemangiomas: PHACE Syndrome

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Abstract

PHACE is a rare congenital anomaly with a spectrum of clinical manifestations described as the cutaneous hemangioma-neurovascular complex syndrome. The main manifestation of PHACE syndrome is the facial hemangiomas and posterior fossa anomalies. The cause and the further development of PHACE have not been elucidated. We report a case with hemangiomas associated with Dandy-Walker syndrome conformed to PHACE, with a complicating delayed neuromotor development after birth.

Key words: Twins, PHACE, Dandy-Walker syndrome, Hemangioma.

Inroduction

PHACE syndrome is a rare neurocutaneous disorder characterized by large cervicofacial infantile hemangiomas and relevant to anomalies of brain, cerebrovasculature, aorta, heart and eyes[1], while Dandy-Walker syndrome is the most common CNS abnormality reported in association with PHACE syndrome[1]. The further effusion of children is still unclear, and the anomalies of the brain may manifest delayed neuromotor development.

Case report

A 1-hour-old female infant was born at term by cesarean section with a birth weight of 2700g, a height of 50cm and a head circumference of 30cm. Her parents and twin-sister were in good health. The pregnancy had been uneventful except an infection of cough in the 6th week gestation. Anomaly of the brain was discovered by ultrasonic examination at the 31st week gestation. An enlargement of 1.4cm width hypoechoic lesions in fetal posterior fossa, a partial missing of the cerebellar vermis, and a channel between the hypoechoic lesions and the fourth ventricle were noted at the obstetric ultrasound. The Dandy Walker malformation was suspected. On the physical examination after birth, a shiny-red hemangioma (2*1*0.5cm) on lower lip was noted (Figure 1A). Elevated lesions of the lip extended to her right upper limb, chest, neck and back when she was 3 days old (Figure 1B). The infant also had a cardiac murmur, which was diagnosed as patent ductus arteriosus by echocardiography. Examination on other systems did not find anything abnormal. Cranial computerized tomography (CT) showed enlarged posterior fossa, partial agenesis of the cerebellar vermis and hemisphere, and a channel between the hypoechoic lesions and the fourth ventricle (Figure 2). The Chromosome and its G zone are normal. The parents refused to perform further genetic work-up. There was no seizure activity or hydrocephalus syndrome noted during 6-month follow-up. However, the neuromotor development may be delayed compared with her healthy sister and other peer children. The infant could not sit or turn back at 6-month of age, and the DDST examination shows that the score was only slightly lower than the normal level (MI<52, DQ<54).

Discussion

Hemangiomas are the most common vascular tumors, occuring in up to 10% of the infants with Dandy-Walker syndrome on the face, head and neck[2]. A newborn with infantile hemangiomas, especially large facial hemangiomas combined with other structural anomalies in a neurocutaneous disorder should be suspected PHACE(S) (OMIM #606519)[3]. The acronym PHACE stands for posterior fossa anomalies (P), hemangiomas (H), artery anomalies (A), cardiovascular system (C) and eye abnormalities (E). Current literature showed that the syndrome may also be complicated by sternal clefting (S) or supraumbilical raphe, heteropia,

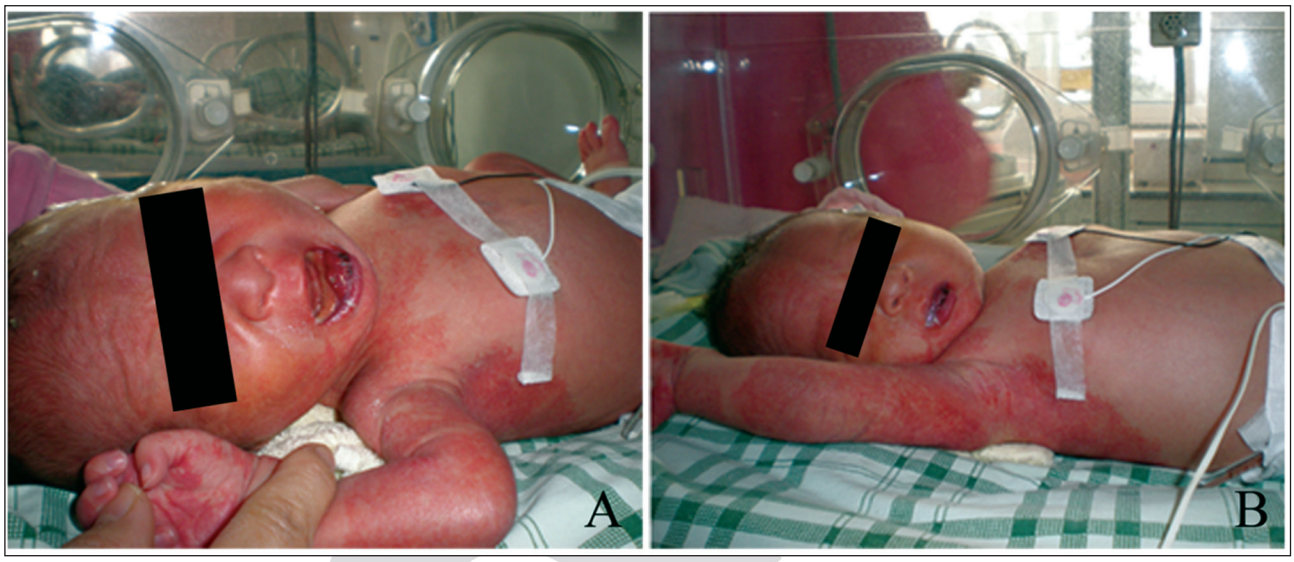

Figure 1. A: Small lower lip hemangioma picture at the birth time of the newborn; B: Large segmental cutaneous hemangioma picture on the 3rd day after the newborn's birth

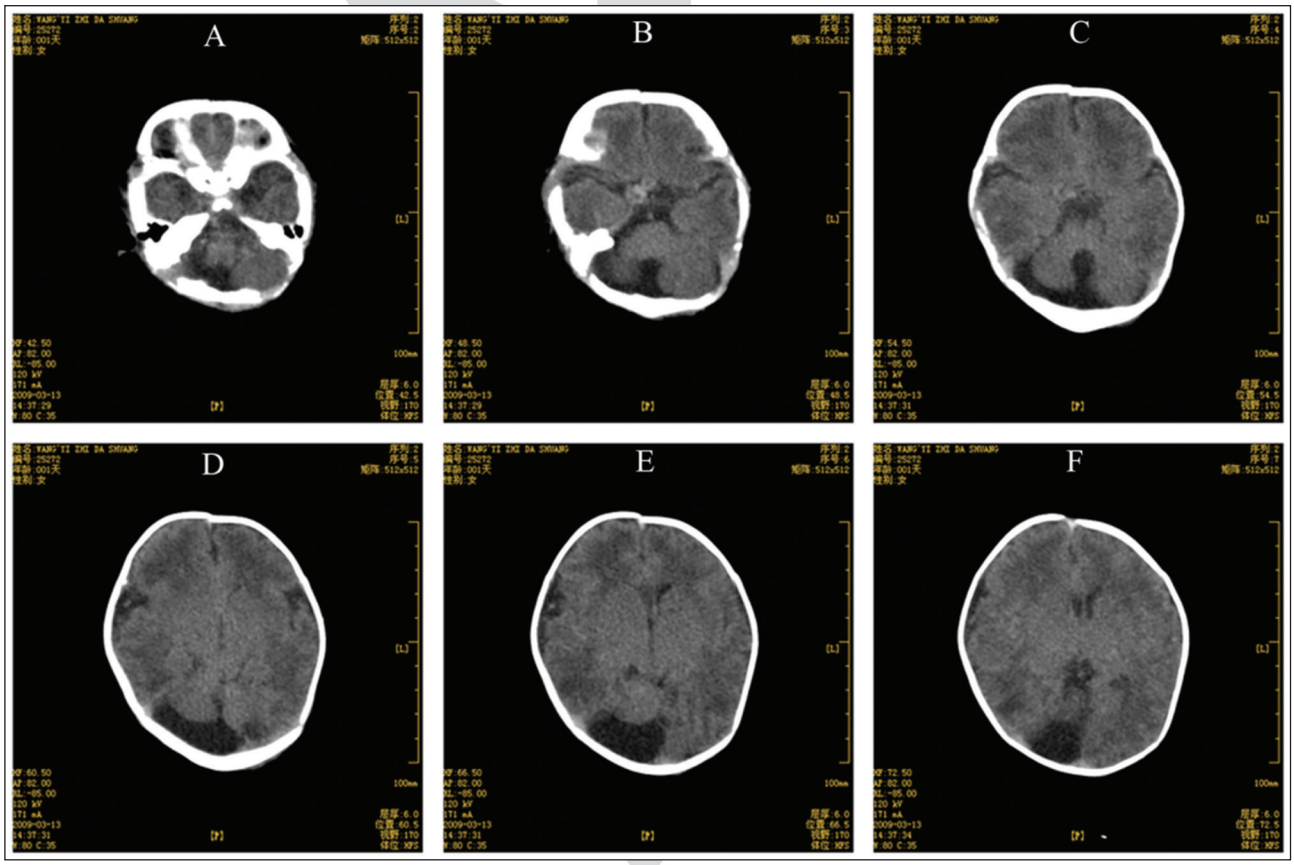

Figure 2. CT scan depicting Dandy-Walker malformation: enlarged posterior fossa, partial agenesis of the cerebellar vermis and hemisphere, and a channel between the hypoechoic lesions and the fourth ventricle

liver, trachea, and endocrine system abnormalities, especially thyroid dysfunction[4]. Almost every person has segmental hemagiomas while only 70% patients have just one extracutaneous malformation of the syndrome[5] . Metry studied the related anomalies and their percentage in 201 previously reported cases with PHACES syndrome: brain 45%, cardiovascular 30%, cerebrovascular 35%, ocular 17%, sternal developmental 26%[6].

The PHACE hemangiomas always involve the orofacial area, and are aggressive with tissue destruction. The hemangiomas have four primary fa-

cial segments: the frontotemporal region (segment 1); the maxillary region respecting the nasomedial sulcus (segment 2); the preauricular region, mandible, chin, and lower lip (segment 3); and the frontonasal region, comprising the medial frontal scalp, nasal bridge, and philtrum (segment 4) [7]. The research reveals a strong correlation between hemangiomas and the anomalies: segment 1 or 4 correlated to structural brain, cerebrovascular, and ocular anomalies; segment 3 correlated to sternal defects and/or supraumbilical raphe[6]. 22% of the patients with PHACE had extracutaneous hemangiomas, which distribute in liver, subglottis, intraoral, pharyngeal, laryngeal mucosa and even trachea[5]. According to the guideline of PHACE, large, plaque-like segmental hemangioma of the face is the core feature, except for some particular cases[8]. In some cases, patients only have small or late-onset hemangioma but with these anomalies, thus still need to be considered as PHACE[9]. In this case, the newborn has small lower lip hemangioma and large segmental lesions in the body. In addition, other findings are suggestive of PHACE. Then the diagnosis of PHACE was hereby confirmed.

Dandy-walker syndrome accounts for 1/3 of the posterior fossa malformations[5]. The incidence rate of Dandy-walker is 1/25000～1/30000 during pregnancy[9]. The Dandy-Walker malformation complex refers to a group of congenital central nervous system malformations involving dilatation of the fourth ventricle, hypoplasia to aplasia of the cerebellar vermis, rotation of the cerebellar vermis and elevation of the tentorium and transverse sinus[10]. The prenatal ultrasound, CT or MRI can diagnose it. Some debates on the ultrasound diagnoses, however, arise recently. A couple of researches revealed that the diagnosis of classic Dandy-Walker is easy and reliable, and it functions effectively from mid-gestation (even as early as the 14th week) by using vaginal sonography, whereas others claimed less stable relationship between the prenatal ultrasonic diagnoses and the pathologic images[10,11]. The etiology of Dandy-walker is still not clear. The malformations may happen in the first 6-7weeks of the pregnancy[12]. The 40 cases of chromosomal analysis performed by Joanna discovered that there were abnormal karyotypes (trisomy 13, partial trisomy 13, trisomy 21, mosaic monosomy X and triploidy) and other normal Chromosomal ones[10]. Our patient had the characteristics of Dandy-Walker identified by the CT and prenatal ultrasonic diagnoses without abnormal karyotype, which probably suffered from malformation related with a cough in the 6th week of pregnancy.

One third to one half of the patients have cardiac anomalies. coarctation of aorta is the most popular anomaly, but other arteries and intracardiac defects can also be reported[8]. According to the literature, there are some cardiovascular malformations: ventricular septal defect, atrial septal defect, pulmonary stenosis, tetralogy of Fallot and so on[13]. There are also a variety of vascular anomalies in this syndrome. Aneurysms, anomalous branches and stenosis of head and neck arteries take place in up to 41% of cases of PHACE syndrome[11]. The sequelae of the PHACE, especially the neurological complication of developmental delay or seizures, occur in up to 90% of those cases with structural cerebral and arterial anomalies[5]. Geoffrey's research suggested a close relationship between the distribution of cervicofacial hemangiomas and the location of intracranial and extracranial vascular (and cardiac) anomalies[12]. The neural crest cells from the posterior diencephalons play an important role in musculoconnective wall connecting with internal carotid artery and great vessels[13]. Therefore, damaged neural crest cells in the diencephalons and the rhombencephalon could cause anomalies of the posterior fossa and the arteries[14].

Our patient has classic features of the two primary clinical manifestations of PHACE (hemangiomas and Dandy-walker syndrome), but has no other anomalies like cardiovascular malformations, ocular abnormalities or sternal defects which have assocation with hemagniomas and also have high incidence in PHACE snydrome[15].

The pathogenesis of PHACE syndrome has not been determined but one theory has been accepted that the essential time is the first 6-8 weeks of gestational age[5]. In this critical period, the gene may have mutation and the neuronal migration (cephalic neural crest cells) could be interrupted. Female predominance leads to the hypothesis of X-linked dominance with lethality in males[16], but a statistically significant difference was found only on structural brain anomalies, which were somewhat more common among male patients[17]. PHACE appears

to occur almost exclusively in singleton pregnancies, though it is possibly pertinent to the greater gestational age[18]. In our case, the accidental cough occurred in the 6th week of gestational age and on the other hand, the twin-sister is a healthy baby. This is the first reported twin pregnancy with one healthy but one PHACE patient so far.

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Coexistence of spinal lipoma, ependymal cyst and dermoid cyst in neighboring localizations in conus medullaris: A case report

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Abstract

Spinal lipomas, dermoid cysts and ependymal cysts are tumoral pathologies associated with a closing defect in the neural tube during the embryological development of the spinal cord. These lesions are quite rarely seen benign tumors of the spinal cord. An intradural intermedullary lipoma at the level of conus medullaris without presence of spinal dysraphism, an ependymal cyst localized intradurally and intramedullary, adjacent to the lipoma proximally, and an intradural and juxtamedullary localized dermoid cyst, adjacent to the lipoma distally, were observed in our case. There has been no previous report of the coexistence of spinal lipomas, dermoid cysts, and ependymal cystic tumors together in one case.

Key words: Dermoid cyst, Ependymal cyst, Conus medullaris, Lipoma.

Introduction

Spinal lipomas, dermoid cysts, and ependymal cysts located at the level of conus medullaris are rare, slow growing, benign tumors occurring due to a defect formed at the time of closing of the neural tube. An ependymal cyst localized intradurally and intramedullary proximal to a caudal lipoma, and an intradurally localized juxtamedullary dermoid cyst distal to the same caudal lipoma at the intradural and intermedullary localization were observed all together in our case. Spinal lipomas without spinal dysraphism are quite rare, and develop through the differentiation of mesenchymal cell residuals into fat cells. Ependymal cysts, which are another rarely seen spinal tumor, develop through persistence of the terminal ventricle, and dermoid cysts form from the ectopic embryonic residuals of the ectoderm. There has been no report in the literature where these three tumoral lesions coexist. All three lesions were resected in our case; no deficits were observed postoperatively in the neurological examination, and the complaints of the patient were regressed.

Case

A 50 year-old woman presented to our outpatient clinic with the complaint of severe pain in her back and waist regions for two years. There were no motor, sensorial and reflex deficits in the neurological examination; neither were urinary dysfunction, radiculopathy, and spinal dysraphism findings present. A tumoral lesion at the T12-L1 level at the localization of the conus medullaris, with identifiable central fat planes at T1 sequences, and a heterogeneous signal feature, and with a cystic component at the superior part and a solid component in the inferior part, was observed in the lumbar magnetic resonance imaging (MRI) (Figures 1A, 1B). An operation was offered and informed consent obtained from the patient. A midline skin incision was performed under general anesthesia in prone position at the level of T11-L2. Total laminectomy and flavectomy were performed to the T12 and L1 laminae. A non-pulsatile and expanded dura was exposed for a vertical segment of 4 cm, at a localization matching the tumoral region. Dura was opened vertically by microsurgical techniques. An intradurally localized juxtamedullary cystic lesion at the caudal part and expanded conus medullaris was observed superior to it. There were no adhesions around the juxtamedullary cystic lesion to the surrounding

tissues and the lesion had a regular wall surface in smudgy yellow color. The cyst was perforated and the easily aspirated cyst content in dirty white color was seen and biopsied for pathological and microbiological examinations. The cyst wall was totally resected after the cystic contents were completely aspirated. A localised intramedullar, yellow, lipomatous mass , neighboring this excised cystic lesion proximally was seen caudate to the conus medullaris. This mass was detected to be attached to the conus and was grossly totally resected. Proximal to these lesions, an intramedullary cystic lesion filled with cerebrospinal fluid was observed. The cyst wall was subtotally resected and the central canal was fenestrated to the subarachnoid space. The layers were closed according to their anatomical order after hemostasis. No neurological deficits were observed during the postoperative period, and the patient's complaint of lumbar pain regressed. Pathological examination of the lesions was reported as a spinal dermoid cyst, a lipomatous tissue, and an ependymal cyst (Figures 2A, 2B, 2C). A milimetrical residual lipoma was seen in the MRI obtained at the postoperative follow-up visit; however there were no cystic lesions (Figure 3).

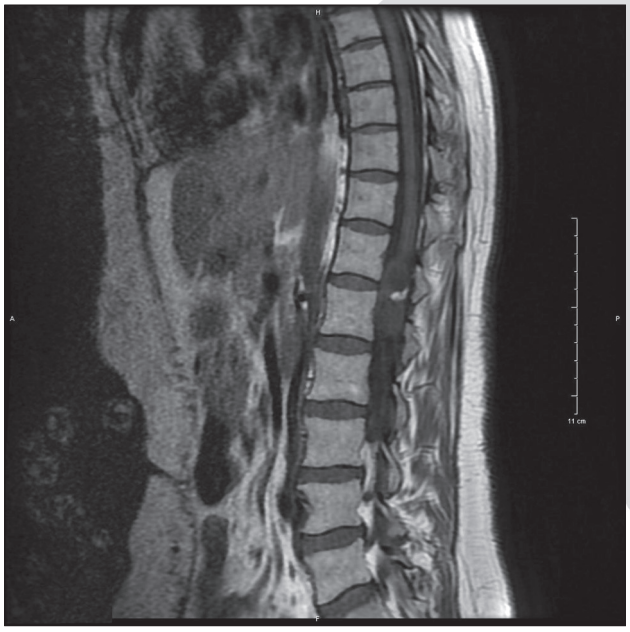

Figure 1A. A lesion with a distinct fatty tissue intensity with heterogeneous signal feature, diffused into a segment of 4 cm. in the localization of conus medullaris at the T12-L1 level, is seen in T1-weighted sequences

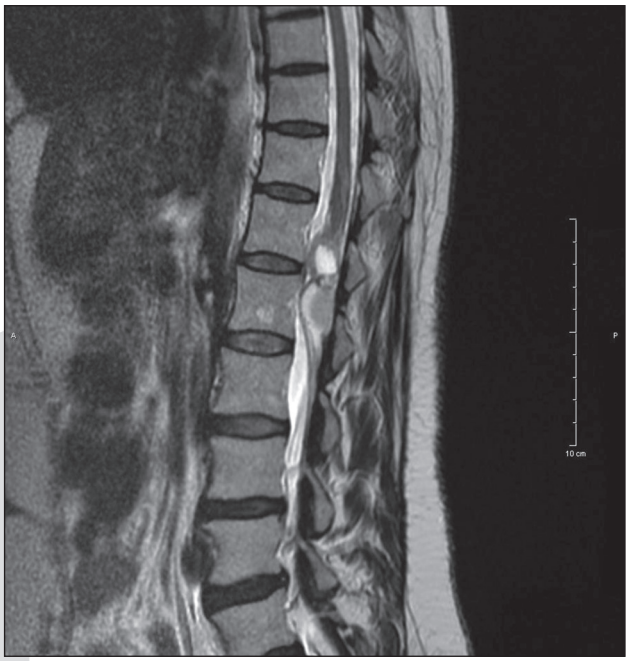

Figure 1B. Superior half is seen as cystic and inferior half is seen as solid in T2-weighted sequences

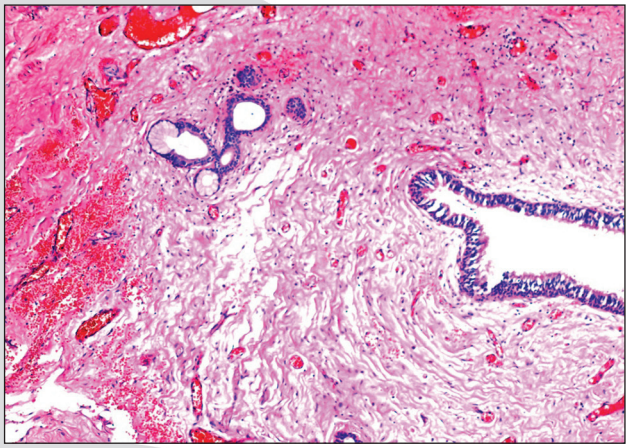

Figure 2A. Dermoid cyst epithelium and seromucosal glands are seen in the subepithelial connective tissue. H&E, 400X

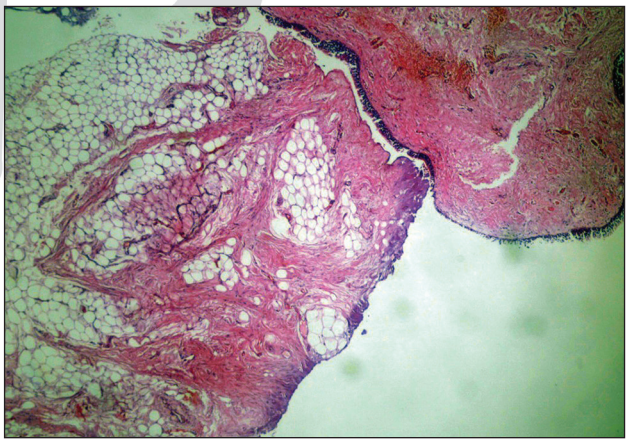

Figure 2B. dermoid cyst and the lipomatous tissue excised together with it are seen. h&e, 400x

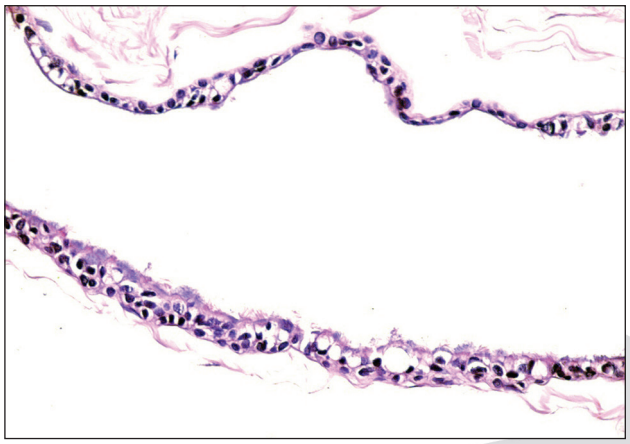

Figure 2C. Basal membrane is not seen in the thin cyst wall corresponding the ependymal cyst; ciliated columnar epithelium is seen. H&E, 400X

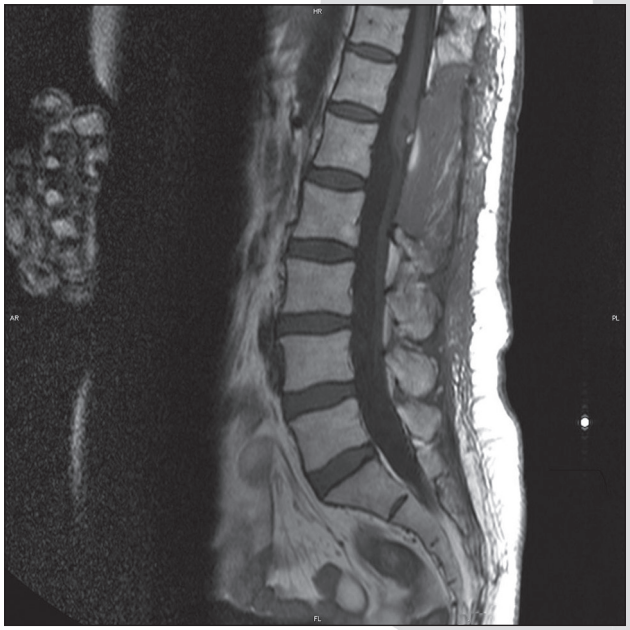

Figure 3. No cystic lesions are detected in the sagittal T1-weighted series, although a milimetrical residual lipoma is observed.

Discussion

Spinal lipomas, dermoid cysts and ependymal cysts are rarely seen, slow growing lesions of the spinal cord. These tumoral lesions occur during the embryological development of the spinal cord due to a closing defect of the neural tube. An intradurally and intramedullaryly localized lipoma at the level of conus medullaris coexisting with an intradural and intramedullary ependymal cyst proximal to it, and a dermoid cyst localized intradurally and juxtamedullary distal to it were observed in our case. When all the case reports on the coexistence of any two of these three tumors without spinal dysraphism are reviewed, it can be stated that this is the first reported case of a spinal lipoma, dermoid cyst, and ependymal cyst, seen altogether, and localized next to each other at the level of conus medullaris without presence of spinal dysraphism (1, 9, 10, 14, 17, 27). Intradural intramedullary lipoma is proposed to develop through the differentiation of mesenchymal cell residuals into the lipid cells; intradural juxtamedullary dermoid cyst is thought to develop from the ectopic embryonic residuals of ectoderm, and caudal intramedullaryly localized lipoma is proposed to cause secondarily the formation of ependymal cystic tumor due to the persistence of the terminal ventricle. Intramedullary lipomas are rather rare tumors which form 1% of all intramedullary spinal tumors (24). Only 0.45 to 0.6% of the intramedullary lipomas occur without spinal dysraphism with a female to male ratio of 1/1 (22, 23). Neurological symptom presentation of intramedullary lipomas without spinal dysraphism and skin lesions generally occurs in the third decade (13). They are most frequently localized at the thoracic region (6), and can expand mostly by longitudinal growth to more than one segment of the spinal cord (12). They do not cause acute spinal cord compression syndrome since they have a soft texture (19).

Spinal dermoid cysts are slow growing benign tumors which are congenital or developed from the ectodermic inclusion residuals (4). These tumors form 0.8 to 1.1% of all primary spinal tumors and are frequently located extramedullaryly or subdurally and juxtamedullary in the conus or cauda equina (4). They are comprised of dermal structures histologically. The contents of the dermoid cysts are generally composed of a soft, sticky glandular secretion in a whitish yellow color (4, 15). These contents are produced by sebaceous glands through the progressive desquamation of the epithelium and transformation of keratin to fatty acids and cholesterol. These tumors are seen histologically as a collagen stroma surrounded by a stratified squamous epithelium. Calcifications may be present in the cyst. Blood vessels are generally encountered in the connective tissue surrounding the tumor; however they do not penetrate into the epithelial wall of the dermoid (4). Malign transformation of spinal dermoids into squamo-

us cell carcinomas have been reported, although rare (18). Differential diagnosis of dermoid cysts by imaging techniques includes teratomas and lipomas due to their high lipid content (10, 20). Patients present with slowly progressing radiculopathy, myelopathy or with cauda equina syndrome. Spinal dermoid cyst contents might rupture into the subarachnoid space and the ventricular system causing hydrocephalus, meningitis or spinal arachnoiditis; while they might be asymptomatic as well. Ruptures can develop spontaneously, post traumatically or postoperatively and carry high rates of morbidity and mortality (3, 5).

Intradural spinal ependymal cysts form 0.4% of all primary spinal tumors (11). Presence of simple columnar or cuboidal epithelium is the characteristic feature of ependymal cysts (11, 16). The cerebrospinal fluid containing cavity surrounded with ependymal tissue in the conus medullaris is named as terminal ventricle or fifth ventricle. Terminal ventricle is a naturally formed step in the embryological development of spinal cord and thus its presence up to the fifth year of life is not pathological (8). Isolated cystic dilatations of the fifth ventricle in adults are assumed by most authors to occur due to the abnormal closing of the connection between the central channel and terminal ventricle by the ependyma and thus the persistence of the lesion (2, 7, 21). Magnetic resonance imaging is the most useful technique in the diagnosis of intramedullary cystic lesions. The T1- and T2-weighted magnetic resonance images of spinal ependymal cysts are well circumscribed, homogeneous, intramedullary lesions with an isointense content with the CSF. There is no contrast enhancement in the lesion. Postoperative follow-up with magnetic resonance imaging is required due to the possibility of recurrence. Cyst fenestration and marsupialisation, and cystosubarachnoidal shunts are among the secure surgical options to prevent recurrence (25, 26).

Conclusion

Dysembryogenetic spinal tumors due to a closing defect of the neural tube are quite rarely seen in adults without the presence of spinal dysraphism. Coexistence of an intradurally and intramedullary localized lipoma at the level of conus medullaris, an intradural and intramedullary ependymal cyst proximal to it, and an intradural juxtamedullary dermoid cyst distal to it are reported in this case report. Theoretically, spinal lipomas occur through the differentiation of the mesenchymal residual cells into the fat cells; ependymal cysts occur with the persistence of the terminal ventricle; and dermoid cysts develop from the ectopic embryonic residuals of the ectoderm. No spinal dysraphism findings were present in our case, and the present lesions caused symptoms in the adulthood in our case. All three lesions were excised in the case; no neurological deficits were encountered postoperatively, and the symptoms of the patient regressed.

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A 57-year-old man with recurrent chest pain, left hemothorax and right hydrothorax

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Abstract

IT is a case about A 57-Year-Old Man with recurrent Chest Pain, Left hemothorax and right hydrothorax, with long time follow-ups, the patient died on the operating table. At last, the diagnosis - cardiac angiosarcoma was confirmed according to the pathological result. Cardiac angiosarcoma is a rarely primary malignant tumor usually begins in the right atrium of the heart or on the pericardium of the heart with average survive time is 6 month. But this patient survived another 8 months when he only received conserved therapy like repeated pericardiocentesis, thoracocentesis, and bleeding control since he first came to our hospital with considerably poor condition. In all, he lived 14 months with acceptable quality of life far more than the average survival time.

Key words: Cardiac angiosarcoma, pericardiocentesis, thoracocentesis, recurrent Chest Pain, Left hemothorax and right hydrothorax.

Introduction

A 57-year-old Chinese man with a 6-month-history of recurrent right chest pain presented to the emergency department of our hospital. The pain often occurred after long time activities, without radiating and can be relieved by 10 to 60 minutes rest and used to be diagnosed as angina. Nitroglycerin didn't work well. The physical examination saw low breath sounds in both pulmonary fields and tenderness below the xiphoid. The heart rate and blood pressure were as normal as other physical symptoms. The laboratorial tests showed anemia, severe liver and kidney dysfunction, negative CK-MB and NT-ProBNP(the specific displayed in the last column in Table1) . Electrocardiogram presented complete right bundle branch block. The cardiac MRI, cardiac CT and aortic CTA illustrated both pleural effusion, hemopericardium, unobvious aortic dissection and some atypical changes.

Three days before, the patient pained intensively and felt blackouts and limbs weakness, followed by loss of consciousness for 20 minutes without convulsion, gatism and hypotension. The diagnosis was vague to the local hospital according to the temporal data of elevated myocardial enzymes, abnormal liver and kidney function (the specific displayed in the second column labeled "7h after syncope" in Table1), liver enlargement, pericardial effusion, ascites and normal cranial CT. As the time by, oliguria and vomit presented and the hemoglobin decreased sharply. When the patient transferred to the upper grade hospital, both pleural effusion and hemopericardium were adverted by thorax CT and right coronary artery morbidity was considered. Then the patient came to our hospital for further diagnosis and therapy.

We adopted pericardiocentesis, thoracocentesis, bleeding control, blood transfusion and some basic drug treatment rather than high risk surgery based on the patient's severe symptoms. Interestingly, we found that left hemothorax but the enffusion from right was clear during thoracocentesis. Finally, the patient was discharged for improved condition with suspected diagnosis as atypical aortic dissection.

The 4 times follow-ups showed that 3 months later, the ultrasonography discovered a 88*51mm irregular shaped and poor acoustic mass at the front of right atrium and this mass growed to 182*141mm on 8th month (figure 1). Such an irregular mixed density mass was also identified by the cardiac PET-CT and cardiac MRI. A benign lesion was considered. On 8th month, the cardiac CT and coronary CTA presented a 3.6cm discontinuous zone at the right atrial groove wall. Regional right atrium communicated with pericardial cavity and right atrial appendage was pressed backward. Right coronary condition was shown in figure 2. Considered right coronary artery and atrial ruptured into the pericardium and led to hemoperi-

cardium, the patient was given the emergency surgery and ultimately died on the operating table (figure 3). At last, the diagnosis - cardiac angiosarcoma was confirmed according to the pathological result (figure 4).

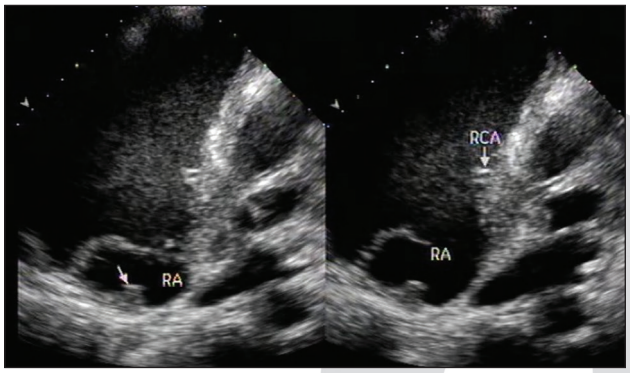

Figure 1. Right front of right atrium presents a 182×141mm irregular shaped and poor acoustic cystic mass within tiny spot flow and a plurality of fiber band, and the cyst wall has multiple heterogeneous echo attachments. Right coronary artery encompassed with hypoechoic area. About 28mm wide echo interrupt between right atrium and cystic mass

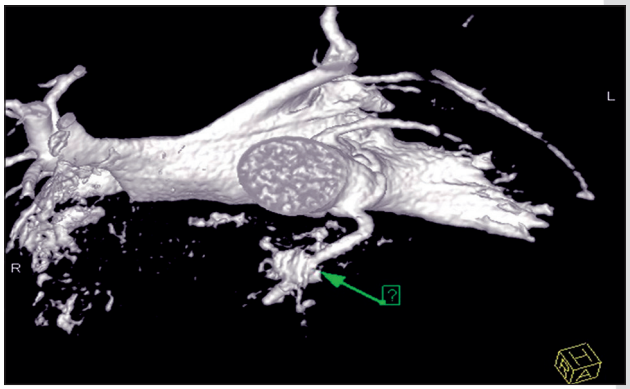

Figure 2. This is a three-dimensional graph of right coronary artery. Arrow refers to the middle junction of right coronary artery around with regional low density shadow and contrast agent irregularly diffuses into pericardium

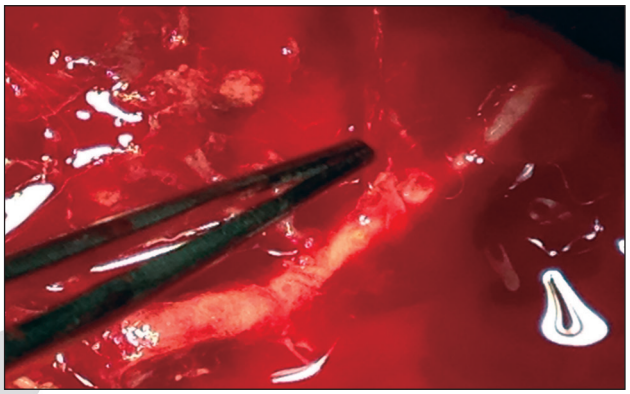

*Figure 3. During the operation, a 2.5*3.0cm impairment could be seen on the front wall of right atrial as well as multiple ejection on the right coronary artery, right atrium and coronary artery rupture*

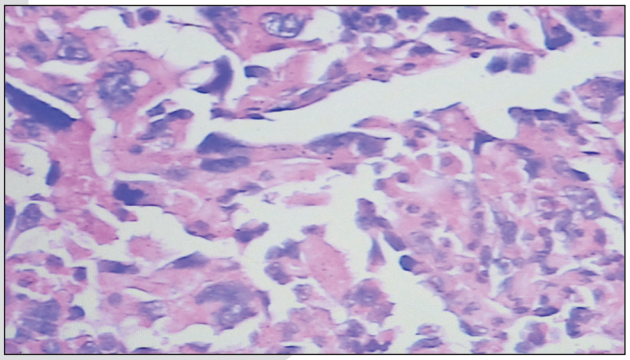

Figure 4. Microscope shows angiosarcoma, and immunohistochemistry shows CD31(++), Vim(+), CD34(+), EMA(-), HHF35(-), HMB(-), CK(-), myogenin(-), Des(-), SMA(-).

Discussion

Cardiac angiosarcoma is a rarely primary malignant tumor usually begins in the right atrium of the heart or on the pericardium of the heart [1]. Nearly 80% arise as mural masses in the right atrium and completely replace the atrial wall at last. These tumors are both symptomatic and rapidly fatal [2].

The diagnosis of cardiac angiosarcoma is often not made preoperatively or even antemortem [3]. The diagnosis of this patient is also confirmed until after death because of the rarity of the lesion and the nonspecific nature of the symptoms and signs. Which also proves that some delicate examination such as cardiac CT, cardiac MRI lack the value in the early diagnosis. During the midanaphase, they can only present a clue for the diseased region but lack of qualitative diagnosis. However, some si-

Table 1. Biomarkers and examinations depend on the time after syncope

Biomarkers or examinations	7h after syncope	36h after syncope	Our hospital 51h after syncope
Hemoglobin (g/L)	107	96	89
ALT (U/L)	1150	851	821
Creatinine (umol/L)	119	434	717
CK-MB (U/L)	75	63.1	12
cTnT (U/L)	2.35	1.17	0.84
LDH (U/L)	1710	3002	1117
Blood amylase (U/L)	146	325	100
ECG	complete right bundle branch block	complete right bundle branch block	complete right bundle branch block
Chest X-ray and Paracentesis	Small volume right pleural effusion	Large volume right hydrothorax and small left pleural effusion	Large volume right hydrothorax and Left hemothorax

Normal value of each biomarker: ALT:0-40 U/L; Creatinine: 53-106umo/L; CK-MB: 0-24 U/L; cTnI: 0-0.1 U/L; LDH:135-225 U/L; Blood amylase:40-220 U/L.

gnals may already want to give us a suggestion before. First, the asymmetric hemothorax indicates that it is a regional lesion and probably is a tumor or a vascular disease. Secondly, the recurrent chest pain after activities that alleviated by rest and the recurrent hemopericardium suggest that the lesion should be located at heart. What's more, the syncope, liver enlargement, ascites and renal failure all show increased preload. Thirdly, the complete right bundle branch block, although not specific, actually accord with the lesion located at the right heart.

Generally speaking, the role of chemotherapy or radiotherapy in the treatment of primary cardiac angiosarcoma has not proven to be beneficial and complete surgical excision is the only mode of therapy that has been shown to prolong survival [4-5]. We consider it as a fluke because he may get these therapies and survive a shorter period if it was diagnosed earlier, because the angiosarcoma in this patient has invaded the heart to such an extent that it is impossible to remove it completely. However, this patient survived another 8 months when he only received conserved therapy like repeated pericardiocentesis, thoracocentesis, and bleeding control since he first came to our hospital with considerably poor condition. In all, he lived 14 months with acceptable quality of life far more than the average survival time.

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First surviving case report of neonatal citrullinemia in China

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Abstract

Citrullinemia is a rare disorder of the urea metabolic cycle caused by a deficiency of argininosuccinate synthetase (ASS). The citrullinemia type I (CTLN1; OMIM no. 215700) present with symptoms during the early neonatal period that include acute hyperammonemia and life threatening encephalopathy. Death usually ensues and no surviving case is reported in China. This is the first report of surviving case of CTLN1 confirmed by ASS gene mutation analysis in China. This case report shows the importance of performing early diagnosis, effectively treated with moderate protein restriction and Arg supplementation, in conjunction with detailed monitoring and follow-up management. The case of classical neonatal CTLN1 had progress normal growth and development at 12 month old now.

Key words: Citrullinemia, Hyperammonemia, Newborn, Argininosuccinate synthetase.

Introduction

Citrullinemia is a rare disorder of the urea metabolic cycle caused by a deficiency of argininosuccinate synthetase (EC 6.3.4.5, *ASS*). Patients with citrullinemia (CTLN) have been classified into three groups: type I and type II, on the basis of residual ASS activity, enzyme kinetics and tissue specificity of the enzyme deficiency. CTLN1 (CTLN type I) was caused by abnormality in *ASS* gene whereas CTLN2 (CTLN type II) was caused by abnormality in the *SLC25A13* gene according to the molecular pathogenesis (1). Citrullinemia type I (CTLN1) includes a neonatal acute (classical) form, a milder late-onset form, a form that begins during or after pregnancy, and an asymptomatic form (2).

Most patients with classical CTLN1 present with symptoms during the early neonatal period that include acute hyperammonemia and life-threatening encephalopathy. Death usually ensues and no surviving case has been reported to date in China. We describe a case of classical neonatal CTLN1 confirmed by biochemical analyses and *ASS* gene mutation analysis, who was treated and followed-up until 12 months of age. This is the first report of a surviving case in China of classical neonatal CTLN1.

Case report

The male infant was born as the first child of nonconsanguineous parents at 40 weeks gestational age after an uneventful pregnancy and delivery. His birth weight was 3,550 g, and Apgar scores were 10 at both 1 and 5 minutes. On the second day of life, he started vomiting, developed hyperhidrosis, and was transferred to our hospital. After admission, the patient was monitored according to standard care and treated symptomatically. However, the clinical situation gradually deteriorated and the infant developed feeding difficulty, became lethargic and had a seizure. On the second day of admission, sudden apnea and coma ensued. The patient was intubated and mechanical positive pressure ventilation was commenced. The rapid change in the patient's condition suggested the presence of an inherited metabolic disease. Metabolic screening was conducted, which revealed hyperammonemia (1,692 μmol/L; normal, 0-30 μmol/L). CTLN was diagnosed on the basis of blood values of citrulline (Cit) (2,513 μmol/L; normal, 2–15 μmol/L) (Figure 1), and massive urinary excretion of Cit [26.75 μmol/mg(Cr); normal,<0.20]. Although treatment was started with a limited protein intake (0.5 g/kg/d)

and adequate calories (75.0 kcal/kg/d) via parenteral nutrition, supplemented by arginine (Arg), sodium benzoate (SB), and carnitine, blood ammonia reached a peak level of 2,110 μmol/L on the third day of admission. The above treatment protocol was maintained, and on the 7th day of admission the patient was extubated due to low ventilation pressure. The blood ammonia levels decreased to 75 μmol/L. The treatment was continued and the blood ammonia decreased further to 25 μmol/L, and blood Cit decreased to 1040 μmol/L on the 12th day of life. The patient regained consciousness, commenced enteral feeding, and was discharged from hospital.

We performed a molecular analysis of the *ASS* gene to further confirm his diagnosis of CTLNI. We sequenced the genomic DNA of each of the 14 coding exons (3-16) of the *ASS* gene in which mutations may occur. The protocol was approved by the Medical Ethics Committee of the Nanjing Maternal and Child Health Hospital, Affiliated to Nanjing Medical University. Written informed consent from the parents of the infant was obtained. Genomic DNA was extracted from peripheral blood leucocytes of the patient and his parents using standard techniques. The PCR amplification of the 14 exons of the *ASS* gene and purification of the amplified fragments were performed as previously described (3). Primers for these exons (Table 1) were designed using an online program Exon Primer (http: //genome.ucsc.edu) with the exception of exon 15 (indicated by an asterisk), which was designed using the software program FastPCR (http: //www.biocenter.helsinki.

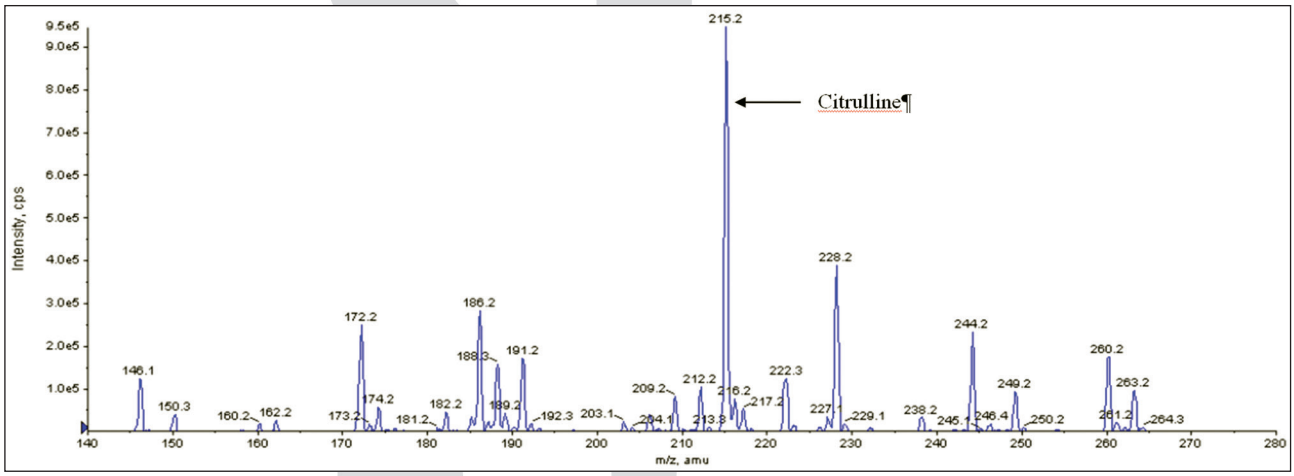

Figure 1. Spectrum of the sample from this patient with citrullinemia [using tandem mass spectrometry (MS/MS)] showing a significant increase in citrulline

Table 1. PCR primers for the amplification of the coding exons of the ASS gene were synthesized by Invitrogen Ltd

exon	upstream primer	downstream primer
3	5’-cactggctgtctcagggtca-3’	5’-gtgagcagacaggctgacaa-3’
4	5’-catgcggatggtgtgaactc-3’	5’-gagcaggatgatctcatact-3’
5	5’-ataggtctcagctagctgag-3’	5’-acaccaggaaaccgctcgag-3’
6	5’-atcctgtggctcctgacagc-3’	5’-gatgctagtgctccctgcta-3’
7	5’-gctctgcagcttacaggcca-3’	5’-ctaggccactggctcagaca-3’
8	5’-gacggacctcacgcgtcctt-3’	5’-tggccgtcctttggaatgag-3’
9	5’-agctgaccctgtctttcctt-3’	5’-atcggcatggagctgctacc-3’
10	5’-tccagggactggtatgtcat-3’	5’-gtaaagagggcctaggttcc-3’
11	5’-ggagtccatatctgtcacat-3’	5’-tgcaacggtccaggctcgtg-3’
12	5’-tgactctgagccttgcggta-3’	5’-agggtctcagggatctctgg-3’
13	5’-gacagtttgggtttcatgcg-3’	5’-ccgagagcctgatagtactt-3’
14	5’-cctccctagtggtatcctgt-3’	5’-atgtcttgaggatccagtca-3’
15*	5’-cagtcctcccttcaagcaga-3’	5’-agcctacccttacacctgg-3’
16	5’-ccaccccagctctgcctgaa-3’	5’-ggaccagggaacaaagctgg-3’

fi/bi/Programs/ fastpcr.htm), which is a free Internet service. Sequencing reactions were performed on amplification products using the Big Dye 3.1 kit (Applied Biosystems, Foster city, CA, USA) and examined on an ABI3130XL DNA sequencer (Applied Biosystems) using sequencing analysis software (Applied Biosystems, Foster City, CA, USA). The sequence analysis showed a homozygous missense mutation of c.970G>A (p.G324S) located in exon 13 in the patient, and the sequencing results from the parents displayed a heterozygous state of the same mutation (Figure 2). Sequence analysis was undertaken in 100 unrelated normal controls.

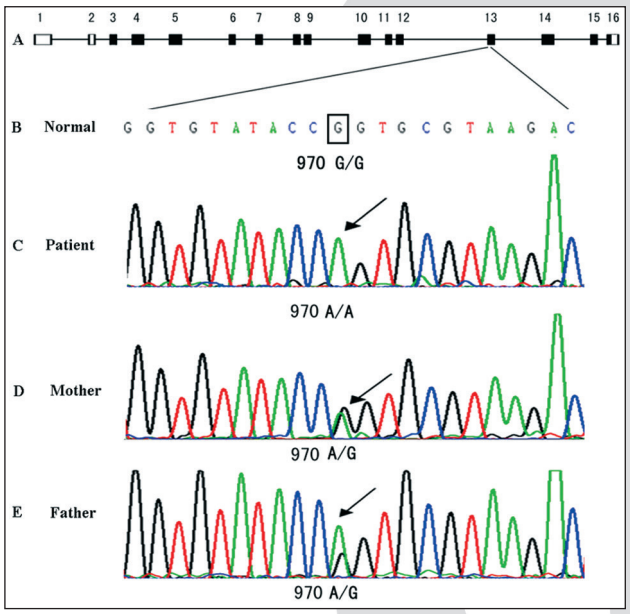

Figure 2. Sequence analysis of exon 13 of the ASS gene. (A) Schematic diagram of the human ASS gene containing 16 exons, 3-16 as the coding region of the exon. (B) Normal ASS gene 970 loci (homozygous G/G). (C-E) A homozygous missense mutation of c.970G>A located in exon 13 was identified in the patient, and the parents display a heterozygous state of 970 loci (G/A).

Special therapy was maintained to reduce the blood ammonia concentration, which included a restricted protein intake (2.0 g/kg/day) by the administration of a formula containing 0.8 g protein and 85 kcal/100 ml. We supplemented with essential amino acids (0.25 mg/kg/day), Arg (500 mg/kg/day) and carnitine (50 mg/kg/d). Vitamins, minerals and trace elements have also been administered to meet the daily recommended requirements. Dietary protein intake was gradually increased, at 12 months of age, the formula was continued and protein intake was increased to more than 3.0 g/kg/day. The blood ammonia concentrations were measured each month or when the infant appeared to have feeding intolerance or became lethargic, which occurred at 1 and at 3 months of age, when blood ammonia levels increased to 212 μmol/L and 254 μmol/L, respectively. After treatment with intravenous SB (250 mg/kg/day) and Arg (500 mg/kg/day), a complete clinical and biochemical improvement (blood ammonia level normalized) occurred within 24 hours.

Follow-up magnetic resonance imaging (MRI) at 1 month of age revealed diffuse high-signal intensity lesions involving the white matter in both occipital lobes and cerebral hemispheres with edematous changes (Figure 3). At 12 months of age, the Gesell developmental assessment measured the infant's skills in personal-social, language, fine motor, gross motor and adaptive areas as 88, 83, 104, 91, and 83, respectively (4). Growth and development data are shown in Table 2, which demonstrates acceptable progress in physical parameters.

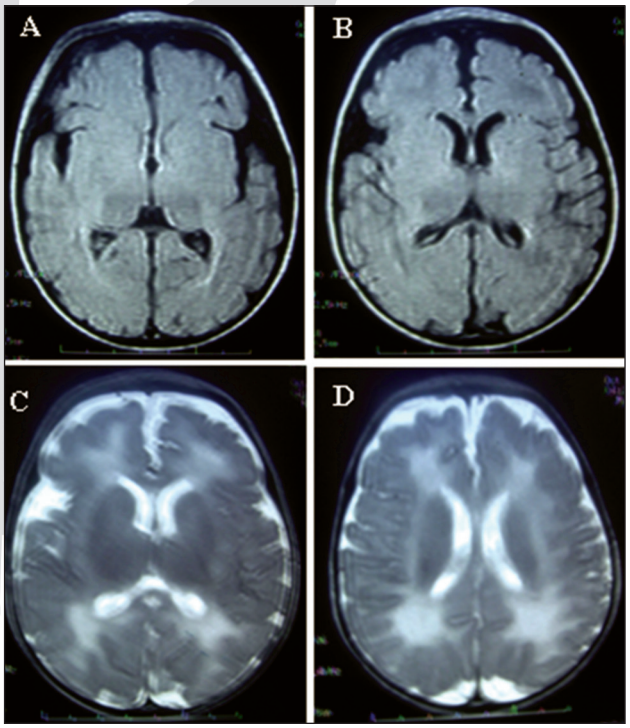

Figure 3. Brain MRI demonstrating diffuse high-signal intensity lesions involving the white matter in both occipital lobes and cerebral hemispheres with edematous changes. (A-B) Hypointensity on T1 weighted image, (C-D) Hyperintensity on T2 weighted image

Table 2. Summary of growth data

Growth data	Birth	3 month	6 month	9 month	12 month
Weight (kg)	3.4	6.1	7.7	9.2	10.1
Length (cm)	49.5	60.2	67.3	72.4	76.1
Head circumference (cm)	34.0	39.1	42.8	44.2	45.1

Discussion

Classical neonatal CTLN1 is a particularly severe condition, which is characterized by very high plasma levels of ammonia and Cit, with death usually occurring in the first week of life (5). Without pre-symptomatic detection, the overall prognosis for classical neonatal CTLN1 has been very poor despite significant advances in therapy (6, 7). Saudubray *et al.* reported on a group of 28 patients treated for citrullinemia due to *ASS* deficiency, 19 of whom died during the first weeks of life and one additional patient died shortly after liver transplantation. Two of the eight survivors were severely disabled (8). Considering the early onset of symptoms in cases of classical citrullinemia, screening samples should be obtained early and a diagnosis needs to be urgently established in the case of feeding intolerance, vomiting or coma (9). Prospective treatments for newborn infants are needed, where diagnoses are made prenatally or before the onset of clinical symptoms, which would be effective in improving survival and preserving normal development (6, 9). Several examples of favorable outcome for prospectively treated infants with neonatal CTLN1 have been reported (10). The recommended protocol for long-term treatment of neonatal CTLN1 requires protein restriction and daily administration of Arg, SB, and sodium phenylacetate or sodium phenylbuterate (11). We describe a patient with a favorable outcome despite a later diagnosis and treatment. However, neonatal screening for citrullinemia and its earliest possible detection and treatment remains the most important approach, and has the potential to improve survival and growth and development (6, 9).

Neuropathological studies in hyperammonemic disorders reveal predominantly astrocytic changes consisting of cell swelling in acute hyperammonemia and astrocytosis in chronic hyperammonemia (12). Acute hyperammonemia leads to increased cerebral blood flow and accumulation of lactate in the brain. Chronic mild hyperammonemia may lead to region selective alterations of the cerebral metabolic rate of glucose. At follow-up MRI of neonatal CTLN, some studies have demonstrated damage to the oligodendro-axonal unit and a decrease in fractional anisotropy in the white matter (13). Our study also reported MRI abnormalities seen in the presence of neonatal CTLN1. It is important to realize that severe brain damage in the neonatal period is not always caused by hypoxic-ischemic disease. Metabolic disorders such as urea cycle defects should also be considered (14).

The *ASS* gene (GenBank No. AY034076) is located in chromosome 9q34.1. To date, 87 mutations have been described in this gene (15). Some of the mutant forms have demonstrated mild clinical symptoms, while others present with severe clinical symptoms and progress very quickly (16). In our case, we found a c.970G>A mutation of the *ASS* gene, which led to glycine-324 being replaced by serine (Figure 4 A-B). The pathogenicity of this mutation is supported by some case reports in USA (17), Korea (18), Turkey, which were associated with severe clinical symptoms, which included feeding difficulty, lethargy, dyspnea, seizure, coma, et al. The cases of mild and asymptomatic type were scarce. This is the first report in China. The crystal structure of human *ASS* protein (glycine-324) was investigated, which came from the Research Collaboratory for Structural Bioinformatics (RCSB) database (http: //www.rcsb.org/pdb/home/home.do), and the 3D molecular viewer of *ASS* (PBD ID: 2NZ2) was analyzed by NTI Vector 10.0.3. The results showed that the glycine-324 of the *ASS* gene is highly conserved in all species (Figure 4C), which was around the alpha helix (α-helix) (Figure 4D). The mutation of c.970G>A (p.Gly324Ser) altered the conservative α-helix structure of the *ASS* protein, impairing its ability to bind to molecules such as Cit and aspartate. The mutation also led to a decline in enzyme activity, resulting in a decreased capacity of the enzyme to effectively play its role in the urea

cycle, which prevented the liver from processing excess nitrogen into urea. As a result, nitrogen (in the form of ammonia) and other byproducts of the urea cycle (such as Cit) build up in the bloodstream. Ammonia is toxic, particularly to the nervous system. An accumulation of ammonia during the first few days of life leads to poor feeding, vomiting, seizures, and the other signs and symptoms of neonatal CTLN.

This case report demonstrates the importance of an early diagnosis, and of effectively treating with moderate protein restriction and Arg supplementation, in conjunction with detailed monitoring and follow-up. The case of classical neonatal CTLN1 described had normal growth and development at 12 months of age. Longer term follow-up and management will be continued.

Acknowledgment

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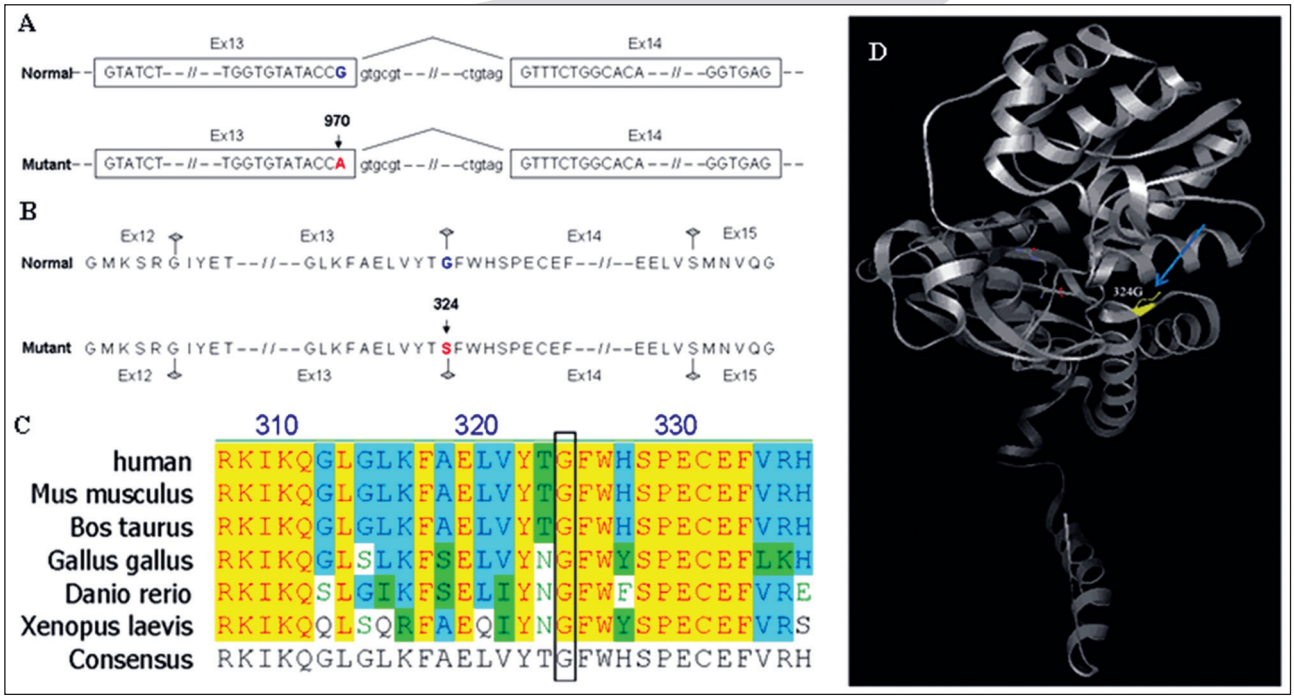

Figure 4. (A) Human ASS protein is highly conserved at amino acid position 324 (which is glycine, indicated by black box) across the different species. (B) G-to-A transition at nucleotide-970 in exon 13 of the ASS gene. (C) Gly-to-Ser mutation at amino acids-324 in exon 13 of the ASS protein. (D) The crystal structure of human ASS protein was done with Cn3D (www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml), the yellow marker is Glycine-324, which was around the alpha helix (blue arrows).

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Three siblings with Laurence Moon Bardet Biedl Syndrome presenting with prolonged cough: A case report

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Abstract

Background and aim: Laurence Moon Bardet Biedl syndrome (LMBBS) is an autosomal recessive syndrome and characterized by hypogonadism, polydactyly, mental retardation, obesity. Familial form of this syndrome is rare. We could not find instances of familial cases presenting with prolonged cough. In this paper, 3 siblings diagnosed with familial LMBBS, a diagnosis rarely reported in literature, were presented.

Case: Three brothers, cases 13, 6 and 3.5 years old and was admitted to our clinic with symptoms of asthma. Clinical follow-up, patients were diagnosed with familial syndromes LMBB.

Discussion: With familial LMBB syndrome cases may present with childhood asthma.

Key words: Familial Laurence Moon Biedl Bardet Syndrome, asthma, childhood.

Introduction

Laurence Moon Bardet Biedl syndrome (LMBBS) is an autosomal recessive syndrome characterized by hypogonadism, polydactyly, retinal dystrophy, and mental retardation, central obesity, and renal anomalies[1]. Obesity has a rate of 90-95%, mental retardation 80-87%, retinal dystrophy 91-93%, polydactyly 70-74%, and hypogenitalism 65-69% [2]. Clinical diagnosis can be put in the presence of at least 4 findings. As far as we know, there are no data to explain the relationship between prolonged cough and familial LMBBS. In this report, 3 siblings diagnosed with familial LMBBS, a diagnosis rarely reported in literature, were presented.

Case report

Three siblings aged 13 (girl), 6 (girl), and 3,5 (boy) presented to Dicle University Pediatric Chest Diseases Department with unremitting wheezing and cough (Figure 1). Other siblings were health. They were a total of 6 siblings and their parents were third-degree relatives. All three children had a body weight > 98% percentile with a normal percentile height for their age. All three children had a history of cough for at least one year, a positive response to short-acting salbutamol, and use of nebule treatment for long period. Blood pressure, pulse rate, respiratory rate were all in normal limits. On physical examination, siblings aged 13 and 3.5 years had polydactyly in both hands, and the other sibling had polydactyly in both hand and feet. All had central obesity and mental retardation that increased with age. Six-year-old sibling had bilateral nephrolithiasis. All had loss of vision increasing with age. Retinitis pigmentosa, an important clinical finding of the syndrome, was marked in the 13-year-old child while other two had retinitis pigmentosa only diagnosed by an electrophysiologic study. No patient had clinical or laboratory hypogonadism or cardiac anomaly. Thirteen and six-year-old cases had both insulin resistance and subclinical hypothyroidism. The youngest sibling had a normal thyroid function and no insulin resistance.

The patients were given nebulizer steroid and salbutamol treatment for bronchial hyperreactivity and referred to plastic surgery for polydactyly. The child with nephrolithiasis was referred to pediatric urology department. The patients with insulin resistance were under follow-up by endocrinology department with age-matched dietary recommendations.

Figure 1. Three siblings with familial LMBBS

Discussion

Bronchial asthma is one of the most common chronic diseases of the childhood. Bronchial hyperreactivity commonly accompanies congenital chromosomal abnormalities such as Down syndrome. Despite it has been reported in the literature that asthma may be seen in the majority of cases with LMBBS[3], the cause-effect relationship is not clear yet and there are no reports in the literature regarding this topic. Three siblings with familial LMBBS had a history of bronchial hyperreactivity present since the birth that responded well to short-acting bronchodilator therapy. They had not used inhaled steroid therapy. Radiologic examinations did not reveal any sign of chronic lung disease in any sibling. A former patient with LMBBS that presented with chronic cough and the present siblings with familial LMBBS complaining of unremitting cough made us think that whether asthma could be included in the diagnostic criteria of this syndrome that has no clinical lung involvement. Polydactyly is one of the major findings of Laurence Moon Bardet-Biedl syndrome and is present in 69% of patients[4]. All three cases we present had polydactyly of hands while one sibling had polydactyly of both hands and feet. Although polydactyly is a common finding, the diagnosis may be delayed until late adulthood when typical phenotypic features of the syndrome do not coexist or do not threaten life. Nevertheless, the disease is commonly diagnosed at a mean age of 3 years. It is interesting that the eldest sibling was diagnosed at a later age (13 years) although all siblings had typical clinical findings such as polydactyly, central obesity, and mental retardation.

Body mass index of the cases are generally greater than 25 kg/m^2 [5]. Our patients had also a body mass index of 26, 28, and 25 kg/m^2, respectively, in descending order of age. Literature data suggest that the risk of hypertension, diabetes, renal and cardiac anomalies increases in this syndrome compared to healthy population[6]. Accompanying renal, cardiac anomalies and diabetes may explain the higher rate of hypertension in this population. Our patients had no renal or cardiac anomalies, however two siblings aged 13 and 6 years had insulin resistance. All had a normal blood pressure.

Previous literature has reported that hypogenitalism is more common among males compared to females. Our cases had normal genital examination findings and gonadotropin levels were within normal limits. Neurological abnormalities in cases with mental retardation may range from cerebral anomaly, gyrus atrophy, and hydrocephalus to no detectable cranial pathology[7]. Mental retardation worsens with age, and together with advanced visual problems it complicates performing daily activities, as in our patients. Visual problems become apparent at a mean age of 6-7; especially nocturnal visual abilities are more severeley affected[8].

Chronic renal failure is present in 30-60% of patients[9]. Severe renal abnormalities may accompany the syndrome in 46% of cases. Common renal anomalies and functional disorder were not present in our cases but the youngest sibling had nephrolithiasis.

In conclusion, apart from the classical clinical symptoms, chronic cough may be the presenting symptom in cases with LMBBS. It should be kept in mind that, in addition to other congenital chromosomal abnormalities, LMBBS may also increase the rate of bronchial hyperreactivity and familial forms of the disease may involve three siblings in a single family.

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Ankylosing spondylitis with increased monoclonal gamma-globulin as initial manifestation

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Abstract

Ankylosing Spondylitis(AS) is a chronic inflammatory rheumatic disease, with a main impact on axial skeleton and the sacroiliac joints. Ankylosing Spondylitis onset is insidious and various, which can be easily misdiagnosed as uveitis, inflammatory bowel disease and other extra-articular diseases. This is the case of a 40-year-old man suspected with the diagnosis of AS but with increased monoclonal gamma-Globulin (IgG,λ) as initial manifestation, which is rarely reported in China. After twice hospitalization in Hematology Department, several times bone marrow biopsy and radiology tests, he was diagnosed as AS at last and received treatment. When dating back his hospitalizaiton, we found that the sacroiliac joint ultrasound results first gave us cue for AS diagnosis. And in the recent follow-up in 2013, the sacroiliac joint ultrasound verified our diagnosis for AS and indicated that the patient illness was under control. For clinicians, more attention must be paid to various onset of AS and the differential diagnosis with hematology diseases.

Key words: Ankylosing Spondylitis, gamma-Globulin, Monoclonal Gammopathy, Ultrasonography.

Introduction

Ankylosing Spondylitis(AS) is a member of the group of the spondylarthropathies and can implicate the entire body. Symptoms such as pain and stiffness in the spine or buttock appear gradually but usually not specific. Extra-articular symptoms such as inflammation of the eye could also happen in AS patients which make it difficult to diagnose AS. And the established criteria inculding clinical symptoms and radiographic classification are not sufficient enough to detect AS in an early stage. Monoclonal gammopathy of undetermined significance(MGUS) is characterized by an accumulation of bone marrow plasma cells derived from a single abnormal clone, which maybe a condition for advancing to malignant hematology diseases, but seldom found in AS patient, especially in Chinese people. The case we report here is an ankylosing spondylitis patient but with increased monoclonal gamma-globulin as initial manifestation, which makes us pay attention to the various onset of AS and the differential diagnosis between AS and MGUS.

Case Report

A 40-year-old man complained of a dull pain for 2 years, worse at night. The pain was generally felt deep in the buttock and in the lower lumbar regions and was accompanied by morning stiffness, improved with activity. The pain cannot be relieved by NSAIDs(Non-Steroidal Anti-Inflammatory Drug), thus he was hospitalized for the first time in our department in January.2010. During this period, his clinical manifestations, increased ESR(55mm/L) and sacroiliac joint ultrasound(abnormal bloodstream signal in sacroiliac joint) supported the diagnosis of Ankylosing Spondylitis, but the pelvis radiology results, HLA-B27 and other autoimmune tests are negative, inconsistent with AS diagnosis. Meanwhile the serum immunoelectrophoresis revealed monoclonal IgG, λ type increased, therefore we considered that he may be diagnosed with hematology disease rather than rheumatology disease and then transferred him to Hematology Department. He underwent bone marrow biopsy and the result was plasma cell 2%. Therefore he was considered the diagnosis of MGUS by hematologists and was discharged. He had a history of tuberculosis but cured. He had no history of smoking, drinking or drug taking. Physical examination finds were no muscle spasms, no inflammatory peripheral

joints, no spinal mobility loss, no flexion or extension restrictions of lumbar spine, no expansion restrictions of chest. The finger-ground-distance was 5cm. The occipital-wall-distance was 3mm. Schber test (-), Patrick test (+), BASFI score 0 and BASDAI score 0.

During the next 2-year follow up, the patient was admitted into Hematology Department twice (January.2011 and August.2011) and our department once (May.2012). Laboratory results during hospitalization (*See Table 1*)

Pelvis radiology results (*See Table 2*)

Discussion

Ankylosing Spondylitis(AS) is a kind of Spondylarthropathy(SpAs) mainly encroaches on spine, ilium, sacrum and five lumbar vertebrae. The prevalence of AS in China ranges up to 0.3%, with the peaking ages for onset in 20 to 30 years old(1, 2)[Sieper, 2002 #1]. Typical clinical manifestations of AS are described in the case, such as buttock and lower lumbar pain, morning stiffness. But extra-articular structures could also happen. About 42% AS patients are presented with at least one extra-articular manifestation. The frequent extra-articular manifestation in AS include anterior uveitis (20-30%), inflammatory bowel disease (5-10%), heart conduction disturbances (3-33%), psoriasis (10-25%), renal abornamalities (10-35%), osteoporosis (11-18%), vertebral fractures (10-18%), etc(3). These extra-articular manifestation could lead us to the wrong dirrection of AS diagnosis. AS 1984 modified New York criteria was the most accepted diagnosis criteria, and the concept of SpAs including AS, reactive arthritis(ReA), Reiter's syndrome, psoriatic arthritis, juvenile onset SpA and arthritis associated with inflammatory bowel disease(IBD), proposed by the European Spondylarthropathy Study Group(ESSG) in 2009

Table 1. Laboratory results during hospitalization

	January 2010	January 2011	August 2011	May 2012	normal range
ESR(mm/h)	55	60	60	45	0-43
CRP(mg/L)	1.21	1.36	2.41	1.47	<3
serum IgG(g/L)	20.2	27.8	22.7	20.6	7-16
serum IgA(g/L)	1.01	1.3	1.02	0.79	0.7-4
serum IgM(g/L)	0.48	0.56	0.42	0.41	0.4-2.3
serum κ LC(g/L)	1.12	1.09	1.03	0.66	1.7-3.7
serum λ LC(g/L)	7.01	7.11	6.6	6.66	0.9-2.1
immunoelectrophoresis	IgG, λ	IgG, λ	IgG, λ	IgG, λ	/
urine κ LC(mg/L)	4.32	9.57	<6.66	15.2	<6.66
urine λ LC(mg/L)	4.67	14.9	<3.66	10.3	<3.66
skull radiology	normal	normal	normal	/	/
bone marrow biopsy (plasma cell)	2%	4.5%	2%	3.5%	/

(LC: Light Chain)

Table 2. Pelvis radiology results during hospitalization

Date	Radiology tests
January 2010	Pelvis CT or MRI: normal Sacroiliac joint ultrasound: abnormal bloodstream signal in sacroiliac joint
January 2011	Pelvis CT or MRI: normal vertebrae MRI: disci intervertebrales inflate in C4-5, C5-6, C6-7, L5-S1, L4-5, with vertebrae cataplasis (*See Figure 1*)
August 2011	Pelvis CT or MRI: left pubic bone chondritis Sacroiliac joint ultrasound: abnormal bloodstream signal in left sacroiliac joint
May 2012	Pelvis CT: blurring version of bilateral sacroiliac joint, cosistent with sacroiliac disease changes (*See Figure 2*) Pelvis MRI: bone marrow edema of left sacroiliac joint.

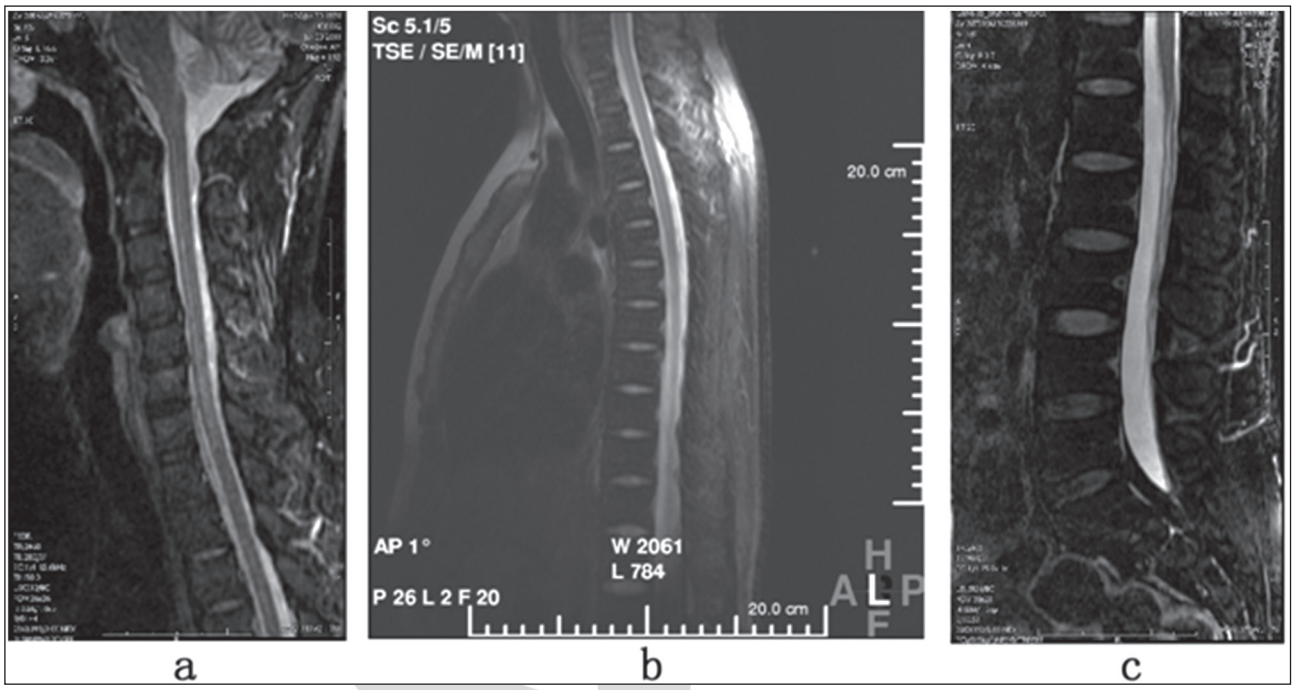

Figure 1. Vertebrae MRI(T2) in January 2011. Lumbar sequence was normal. C4-5, C5-6, C6-7, L4-L5, L5-S1 disc bulged, oppressing the dural sac, with vertebrae and local small-joint osteoarthritis. There were no yellow ligament

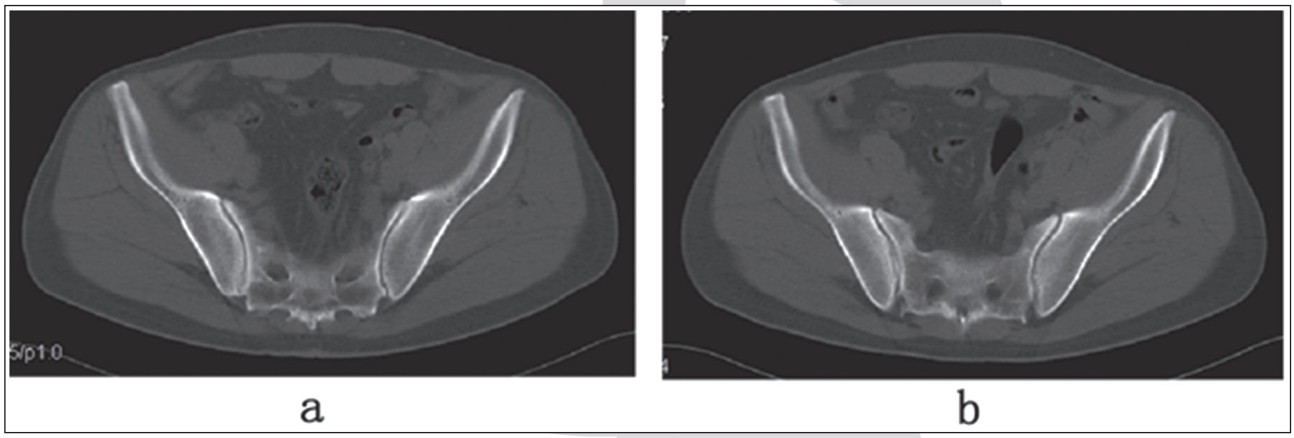

Figure 2. Pelvis CT in January 2010 and May 2012. It was normal in January 2010. But in May 2012, there was articular degeneration on bilateral sacroiliac joint, consistent with sacroiliac disease changes, with ankylosing spondylitis to be considered

can also be mirrored for the diagnosis of AS. There is a frequency up to 90% of HLA-B27 in AS in China. Increased ESR(erythrocyte sedimentation rate), CRP(C-reactive protein) are aided to making diagnosing decision, though they are unhelpful in monitoring the response to treatment (4).

AS with increased monoclonal gamma-Globulin as initial manifestation is rarely reported in clinics in China. Although it was reported in Renier G's research of 557 patients from 1960 to 1990, monoclonal gammopathies were found in 7 patients, 5 with IgG type and 4 with λ type, patients were all already definitely diagnosed with AS(5). Veys EM(6) also reported increased IgG, IgA and IgM serum levels in patients with AS. However, only increased IgA is prevalent and meaningful in AS patient. Franssen MJ and Cowling P(7, 8) worked out the study in 1980s to prove serum IgA levels, but not IgM and IgG, correlated most frequently with chest expansion, lumbar flexion, inflammatory activity, extensive radiological changes and a composite index of disease

activity(IDA). Seldom do we make differential diagnosis between AS and monoclonal gammopathies. Monoclonal proteins(or M-proteins) are individual antibodies produced by plasma cells arising from a single clone of cells. Each M-protein consists of two heavy polypeptide chains and two light polypeptide chains. Different kinds of M-proteins are designated by heavy chains by IgG, IgM, IgA, IgD, IgE, or by light chains or by κ or λ. The most component of M-protein is isotype IgG, for about 69%(9). MGUS is one part of monoclonal gammopathy and accounts 2/3 for it. According to the 2010 International Myeloma Working Group(IMWG) guidelines, MGUS is defined as having serum M-protein<30g/L, clonal plasma cell(PC) population in bone marrow(BM)<10%, and absence of end-organ damage including hypercalcemia, anemia, renal insufficiency, polyneuropathy, bone lesion, heart failure, gastrointestinal involvement, soft tissue involvement and light chain deposition disease(LCDD) (10). The prevalence of MGUS using serum protein electrophoresis is estimated as 3% to 4% in persons older than 50 years. But there was no data for persons younger than 50 years old. MGUS can be considered as a premalignant state. If serum M-protein is over 30g/L or clonal PC population in BM is over 10% or with the evidence of Ben-Jones protein and bone destruction, the patients have higher risk of developing multiple myeloma(MM), Waldenstrm macroglobulinemia(WM), light-chain amyloidosis(AL) or related conditions. In a follow up of 241 patients diagnosed with MGUS study, 19% are binary monoclonal gammopathy while 24% turn to MM, WM or AL(11). IgG, λ is related to POEMS syndrome(polyneuropathy, organmegaly, endocrinopathy, M-protein and skin changes syndrome) and scleromyxedema, and M-protein≥15g/L also has the risk of 21% of progression to MM. Conditions such as osteoporosis, hip fractures, and peripheral neuropathy are also associated with MGUS, which needs to be differentiated with AS.

The patient's onset age, clinical manifestation, physical examination and increased ESR are consistent with AS diagnosis criteria, however the negative HLA-B27, especially sacralis radiology results, swayed our mind for AS. Moreover, the patient has serum IgG, serum λ, urine κ, urine λ increased, inevitably, we have to consider the possibility of hematology disease. Especially as the patient has serum IgG,λ increased over 15g/L, considering the possibility of malignant disease, it is necessary to get further suggestion from hematologists. The patient repeatedly has serum IgG,λ increased and PC population in BM <10% during two hematology department hospitalization, referring to the diagnosis criteria of MGUS proposed by IMWG, the diagnosis of MGUS is no doubt. But for the diagnosis of AS, thanks to the pelvis radiology in May 2012, we could finally make the diagnosing decision of AS.

The insidious and various onset of AS make it difficult to diagnose definitely just by acquiring clinical manifestations. Most of the time, we need to turn to help for radiology tests. However, it may take several years to progress to radiological sacroiliitis. When the radiology evidences are irrefutably for AS diagnosis, the patient may have lost the best opportunity for treatment. The most earliest changes in sacroiliac joint(SIJ) of blurring cortical margins of the subchondral bone on plain radiography, even on CT(Computerized Tomography) or MRI(Magnetic Resonance Imaging), can be neglected by careless radiologists. To sum up, because of the incomplete established classification criteria for AS, which rely on the combination of clinical symptoms plus unequivocal radiographic sacroiliitis of at least grade 2 bilaterally or grade 3 unilaterally, and the defect of pathognomonic clinical feature or laboratory test to make the diagnosis, there are still a number of AS diagnosis were delayed. It is still a challenge to attempt to identify the reason for low back pain. A more precise radiology method for detecting early changes for AS is needed. Ultrasonography has now proved a highly sensitive, noninvasive, and practical tool in assessment of bone and joint pathology, which can also be used to assess treatment response(12), and Zhu JA(13) have made the first time exploration in this field in chinese mainland. Thanks to the good cooperation with department of ultrasound in our hospital, many patients of AS in our department are able to receive early diagnosis and treating methods. Moreover, few AS patients may undergo pulmonary fibrosis. The patient coincidently has the pulmonary fibrosis radiology manifestation in the first time chest X-ray. But we cannot say

whether it can be used for the consideration of AS, as he also has the history of tuberculosis.

In 1992, Quinton R(14) reported a similar case of a patient with 10 years of MGUS and 44 years of AS developed a nephrotic syndrome secondary to renal amyloidosis, thus in the next follow up, except for serum monoclonal gamma-Globulin and bone marrow, the patient also needs to check 24-hour urine protein and serum creatinine to get his renal function evaluated. What's more, according to IMWG suggestion, the present approach to a patient with MGUS is a prudent "watch and wait" strategy that monitors patients on the basis of their risk category. Approximately 40% of all MGUS patients are with low-risk. Therefore, the patient should be followed with serum protein electrophoresis, baseline BM examination and skeletal radiography in 6 months and, if stable, every 2-3 years or when symptoms suggestive of a PC malignancy arise. We finally choose the treatment of DMARD(Disease Modifying Antirheumatic Drug) consisting of sulfasalazine thalidomide, paeoniae and alba for him. In the follow up in January.2013, the patient's condition was: ESR 37mm/h, CRP 0.74mg/L, serum IgG 22.1g/L, serum IgA 0.41g/L, serum IgM 0.79g/L, serum κ LC 0.72g/L, serum λ LC 5.76g/L, 24-hour urine protein 0.02g/24-hour. Pelvis CT or MRI: normal. Sacroiliac joint ultrasound: abnormal bloodstream signal in sacroiliac joint. One more time biochemistry tests and radiology exams verified our diagnosis for AS and indicated that the patient illness was effectively controlled.

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Late onset recurrent seizures in post-stroke patients potentially induced by intermittent theta burst stimulation

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Abstract

Seizure induction is one of the most serious adverse effects of repetitive transcranial magnetic stimulation (rTMS). It can occur in non-epileptic patients or even in healthy subjects under conventional or patterned rTMS such as theta burst stimulation (TBS). Currently, there are no safety guidelines for TBS. It is important to consider as well, that besides safety guidelines, the risk of having a seizure as a consequence of TBS also depends on the features of each individual patient. At present, we have not found in previous studies any case report on seizures induced by intermittent theta burst magnetic stimulation (iTBS). We report here recurrent seizures induced by iTBS transcrainal magnetic stimulation in two stroke patients. The multiple sessions of iTBS heightened seizure susceptibility associated with epilepsy. The TBS parameters combination should be carefully chosen to make sure that they are within safe limits.

Key words: Theta burst stimulation, seizure, transcranial magnetic stimulation.

Introduction

Seizure induction is one of the most serious adverse effects of repetitive transcranial magnetic stimulation (rTMS).[1] It can occur in non-epileptic patients or even in healthy subjects under conventional or patterned rTMS such as TBS, as it has been described for the first time by Oberman and Pascual-Leone.[2] Safety guidelines originally proposed and recent reviews by the International Federation of Clinical Neurophysiology defined the parameters of what could be considered a safe protocol for rTMS.[3] It is important to consider as well, that besides safety guidelines, the risk of having a seizure as a consequence of rTMS also depends on the features of each individual patient. At present, we have not found in previous studies any case report on seizures induced by intermittent theta burst magnetic stimulation (iTBS) Here, we report recurrent seizures induced by intermittent theta burst stimulation (iTBS) transcrainal magnetic stimulation (TMS) in two stroke patients.

Cases report

The first patient was a 33 year old right-handed man. Right basal ganglion and temporal lobe were affected due to cerebral hemorrhage in this patient. Intracranial hematoma was removed through a surgery. He suffered from a severe visual spatial neglect accompanied by depression following the surgery. He received two-week excitability iTBS over left dorsal lateral prefrontal cortex (DLPFC) 2 months after cerebral hemorrhage. The study was approved by the local Ethical Committee with the purpose of exploring the efficacy of intensive rehabilitation combined with theta burst magnetic stimulation over the left prefrontal cortex in stroke patients with unilateral neglect. He had no history of seizures or febrile seizures, no family history of epilepsy. He just took felodipine 5mg once daily to control hypertension.

We were delivering TMS with a Magstim Rapid 2 Stimulator delivering biphasic pulses via a butterfly coil (87mm outer diameter of each loop). The coil was held in a posterior position with an approximate angle of 45 deg from the midline. We were targeting F5 as the left DLPFC according to the international 10/10 EEG system. Stimulation intensity was set at 80% of resting motor threshold of the contralateral resting abductor pollicis brevis muscule, by the method of limits following the guidelines approved by the International Fede-

ration of Clinical Neurophysiology.[4] We used the intermittent TBS protocol in which 10 bursts of high-frequency stimulation (3 pulses at 30Hz) was applied at 5Hz every 10s for a train of 600 pulses, including 4 trains every 15 minutes in a single day. Stimulation lasted for 14 consecutive days. The fist seizure occurred at home 2 months after the final stimulation. The patient presented a secondarily generalized tonic-clonic seizure. He started with a slow developing flexor contraction of his left hand and loss of consciousness, followed by clonic contractions. Urinary incontinence was observed. Total duration was approximately 5 minutes. The seizure ended when the first-aid personnel reached the home. A postictal confusional state lasted for approximately 20 minutes, during which time patient was unable to answer simple questions and showed disorientation. He could not remember what happened to him. The presentations and slow recovery, including the postictal state, were the characteristics of a real seizure. There were no newly abnormal neural signs during clinical and neurological examination in emergency room. The biochemical parameters were within normal range, and focal or generalized epileptiform discharges were not found in electroencephalogram. The neglect symptoms and depression ameliorated significantly. Seizures could not be controlled by Valproic acid (Depakine, 500 mg, twice daily). Seizure occurred every one month during one-year follow-up on average. Oxcarbamazepine 300mg and Phenobarbital 60mg both twice daily was used recently. Meantime, Depakine decreased to cease ultimately. The seizures were controlled for 3 months until now.

The second patient was a 63-year-old woman. The ischemic stroke affected the right middle cerebral artery territory 2 weeks before she received the same iTBS protocol. She presented left hemiplegia and left spatial neglect. No seizures were referred by the patient or her relatives. The first seizure occurred at home 1.5 months after the final stimulation. The patient presented a secondarily generalized tonic-clonic seizure. After the second episode, Carbarmazepine (Tegretol) 200mg twice daily was used to control the seizures. At one year follow-up, she had no more ictal activity.

Discussion

The clinical diagnosis was considered as TBS-related seizures in the two patients. To our knowledge, this may be the first report about the TBS-related seizures that occurred repeatedly after intermittent theta burst stimulation. TBS was generally regarded as a safe and long-lasting powerful tool for motor and prefrontal cortex physiology and behavior.[5,6] One session of repeated (4 trains) continuous TBS one day could induce long-term improvement of visual neglect with no adverse effects.[7] The multiple sessions of iTBS could be seizure-induced when 4 trains were applied daily during 14 consecutive days. The multiple sessions of iTBS protocols appear to lead to sustained changes in cortical activity lasting well beyond the duration of the stimulation, providing a putative index of underlying long-term potentiation (LTP) process that can be recorded in vivo from human cortex. Currently, there are no safety guidelines for TBS. The one incident of seizure induced by cTBS was described by Oberman and Pascual-Leone in a 33 year old healthy man without risk factors for epilepsy.[2] The seizure occurred following about 50 bursts (10s) of TBS to the primary motor cortex at an intensity of 100% of resting motor threshold. It highlighted the need for an intensity-dosing study with TBS protocols to assess the seizure risk. No study followed up with participants in the subsequent hours or days following TBS, and thus only immediate effects could be estimated.[8] In our study, the two stroke patients with multiple sessions of iTBS (4 trains every 15 minutes daily) presented recurrent seizures after the subject left the laboratory. When it was decreased to 2 trains of iTBS daily, there was no recurrent seizure in another 10 stroke patients for at least 3 months follow-up. It was reasonable to conclude that multiple sessions of iTBS heightened seizure susceptibility associated with epilepsy. Additionally, the cases we reported here also highlighted the need of assessment of total number of pulses or duration with TBS protocols for the risk of seizure. At present, TBS, both intermittent and continuous, should be applied with caution and appropriate precautions should be taken, including physician supervision and emergency medical care access. The TBS parameters combination should be carefully chosen to make sure that they are within safe limits.

Additionally, we make a hypothesis that multiple sessions of inhibitory cTBS may chronically control refractory epilepsy due to excitability iTBS leading to repeated seizures. More experiments should be conducted to test the hypothesis.

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Depression and breast cancer

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Breast cancer is the second more frequent type of cancer in the world and the most frequent among women. Every year, near 22% of all new cases of cancer in female patients are breast cancer[1]. It is the most frequent type in developed and emerging countries.

Almost 1.4 million cases of this cancer were expected in 2008 worldwide, what represents 23% of all types of cancer[2]. In Brazil, the National Cancer Institute (INCA – Instituto Nacional do Cancer) estimates that 52,560 new cases will be diagnosed in 2012, with an estimated risk of 52 cases every 100,000 women[1].

The strongest risk factors for breast cancer are female gender, age and family history are. This kind of malignancy is usually a result of a complex interaction of many factors.

Breast cancer is also one of the main causes of death in women. The medium survival in five-year is 61%, and it ranges from 57% in the emerging countries to 73% in developed countries. In Brazil, mortality rates for breast cancer are still high; probably due to late diagnosis. In 2010, for example, the number of deaths among breast cancer patients was 12,852 (147 in men and 12,705 in women)[1].

Every type of cancer is associated with specific burden to patients, and breast cancer interferes with physical and psychological aspects of women. There is also a considerable impact related to surgical and adjuvant treatment on mental health of women affected by breast cancer[3].

After knowing the diagnosis, many patients face different personal conflicts. They struggle in accepting the disease, feel insecure about the treatment and future, fear social and family discrimination[4]. Besides, they often present emotional instability and self-image distortion. The latter manifests as self-steem and sexuality problems.

Breast cancer occurs in a psychosocial context that includes many aspects, which can influence women's reaction to diagnosis, treatment and survival. Functional characteristics, daily stress and other disorders not related to cancer can drastically affect psychological function, either due to increased vulnerability because of cancer or as a result of premorbid factors, like personality. These characteristics seem to be stronger risk factor for depressive symptoms than disease-related variables[5].

Although the risk factors are not the same, the diagnosis of cancer usually represents an emotional burden and, thus an axis IV diagnosis according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorder)[6]. In this context some psychiatric syndromes can occur: adjustment reactions, even affective disorders (mainly depression), anxiety or psychosis. These disorders also represent a complex interaction of risk factors, such as: personality traits and coping strategies[3].

More than half of patients with breast cancer have a psychiatric disorder, specially depression and anxiety [7,8]. The prevalence, however, vary among different studies. Spiegel and coleagues[8] report taxes that range from 4.5 to 50%. Other studies show variation between 3 and 55%[9,10,11]. These studies usually include all depressive disorders and not only major depressive episode[12]. The incidence of psychiatric comorbidity is also influenced by the phase of the treatment[13] (chemotherapy, surgery, radiotherapy).

Like cancer, depression is also considered a public health problem and one of the main predictors of mortality and disability[14]. When untreated, depression is associated with poor cancer treatment adhesion, increased length of hospitalization and worse morbidity of the breast cancer[15,16].

Major depression represents 4.4% of all diseases, numerically similar to ischemic heart disease or diarrhea[12]. The peak incidence of major depression is the fourth decade of life, but the first episode can occur at any time. Patients with an early

onset of the first episode had more severe disease, increased recurrence, psychiatric comorbidities and clinical impairment [17].

Depression as defined here should not be understood as normal sensation of sadness, disthymic disorder, mood disorder induced by psychoative substance or related to a general medical condition (stroke, hypothyroidism) or grief. Periods of sadness are part of the human being and should not be misdiagnosed as major depressive episode, unless are satisfied criteria of severity, length and impairment [6].

An important feature of a major depressive episode is a distinct period of abnormal mood (depressed) or loss of interest in virtually every activity. One should also experience at least four of the following symptoms: changes in appetites or weight, energy loss, sleep disturbance, decreased motor activity, guilty, impaired thoughts, difficulties in getting concentrated or recurrent thoughts about death or suicidal ideation. The symptoms should persist during most part of time, days during at least two consecutive weeks. There must be impairment in social, professional functioning or other important areas of the patient. The major depressive disorder is a clinical course characterized by one or more major depressive episodes[6].

Most of the depressive episodes reach complete remission, either spontaneously or with treatment. The episodes can last months or even a year[18]. Persistent major depression, which lasts more than two years, is present in almost 20% of patients and chronification can occur regardless of the improvement with treatment [19].

Psychosocial factors, particulary personality and social variables are more frequently associated to depression in breast cancer. Although characteristics of the tumor and it's treatment are cited, they're not always identified as risk factors for mood disorders in those patients [5]. The risk factors are the same associated to depression and anxiety in general population: Young age, previous psychological problems and poor social support[3].

The methodological variation, different instruments to assess depression, different cut points for diagnosis contribute to the huge discrepancy in the current findings. In general, the more specifically "depression" is defined the lowest rates of depression are reported and association with cancer lacks statistical significance. Although the association between depression and cancer is widely studied, many articles fail in demonstrating the relationship between depression and aspects of the cancer itself, what suggests that risk factors for depression in those patients are more related to the patient and not to the disease or its treatment [3,5].

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Instructions for the authors

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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.

Introduction

In order to effect high quality of Papers, the authors are requested to follow instructions given in this sample paper. Regular length of the papers is 5 to 12 pages. Articles must be proofread by an expert native speaker of English language. Can't be accepted articles with grammatical and spelling errors.

Instructions for the authors

Times New Roman 12 points font should be used for normal text. Manuscript have to be prepared in a two column separated by 5 mm. The margins for A4 (210×297 mm2) paper are given in Table 1.

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Paper size	A4
Top margin	20 mm
Bottom margin	20 mm
Left margin	20 mm
Right margin	18 mm
Column Spacing	5 mm

Regular paper may be divided in a number of sections. Section titles (including references and acknowledge-ment) should be typed using 12 pt fonts with **bold** option. For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option. Figures should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. Figures must be a resolution of 300 DPI, saved in TIFF format, width 10 cm min. For the figure captions 12 pt *Italic* font should be used. (1)

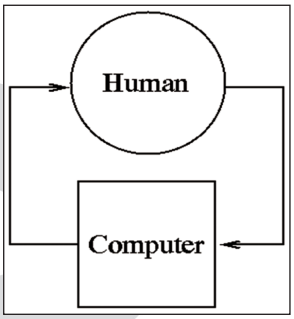

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

References

1. *Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341: 1284–1291.*
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