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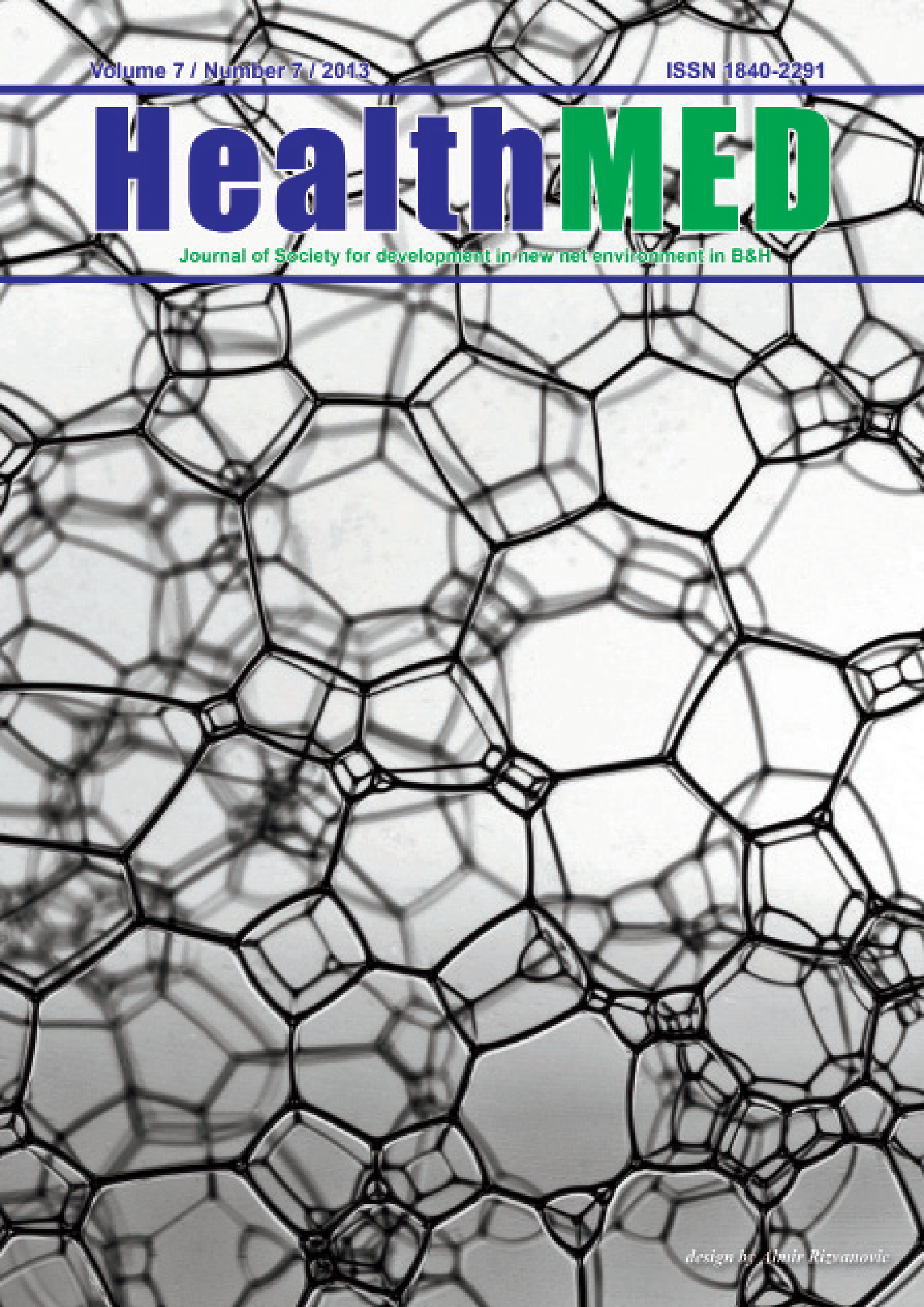

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Sadržaj / Table of Contents

Sadržaj / Table of Contents

Sadržaj / Table of Contents

The inhibitory effect of Silymarin on Cell viability and cellular COX-2 and iNOS level in HepG2 cell line

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Abstract

COX-2and iNOS are key enzymes in inflammation and their up regulation in many types of cancers has been reported previously. Hepatocellular Carcinoma is a prototypical inflammation-associated cancer, and COX-2 and iNOS gene expression increase in this condition. In order to develop anti-cancer drugs, natural chemical are of interest because of their low toxicity and side effects. Among them, silymarin is well known as a hepatoprotective as well as anti-cancer drug. Immunomodulatory effects of silymarin vary among different cells and its effect on COX-2 and iNOS level in HepG2 cells remains unclear till now.

To determine HepG2 cells viability as well as COX-2 and iNOS level in presence of silymarin. IC50 of silymarin were determined for HepG2 cell proliferation by MTT assay and then cell viability as well as their COX-2 and iNOS level in the presence of 50, 75 and 100µg/ml silymarin was measured.

Our results showed that silymarin significantly decrease cell viability in 12 and 24hr intervals while significant decrease in COX-2 and iNOS level were just shown 24hr after treatments($p<0.05$). There was no significant correlation between COX-2 and/or iNOS level and cell viability in treated HepG2 cells. Silymarin probably affect cellular COX-2 and iNOS level at their gene expression level in HepG2 cell lines.

Key words: Silymarin, HepG2, COX-2, iNOS.

Introduction

HCC which ranks among the most common malignancies worldwide often occurs as a secondary condition to chronic hepatitis and it is a prototypical inflammation-associated cancer [1]. Inflammatory reactions are triggered in many liver diseases, as the consequence of the introduction of a toxin, drug or infectious agents. Inflammation occurs to induce a repair process and to reestablish the original functions of the hepatic tissue [2]. The precise control of inflammation is essential for the prevention of chronic inflammatory disorders, as well as for inhibiting the exacerbation or progression of diseases, including many types of cancers [3].

COX-2 and iNOS are key enzymes in inflammation and oxidative stress, respectively [4]. Both COX-2 and iNOS are inducible enzymes, mediating the similar pathological processes [5]. Previous studies showed that improper up-regulation of COX-2 and/or iNOS had been associated with pathophysiology of certain types of human cancers including prostate, breast, gastric, pancreatic, lung, head and neck, bladder, colon cancers as well as HCC [6-13]. An analysis of the previous literatures suggested that iNOS, and products from COX-2 pathways were involved in the regulation of several biologic processes responsible for tumor growth, such as host immune response, proliferation, resistance to apoptosis and tumor angiogenesis [14,15].

Many chemicals with cancer preventive effect have been of interest in recent years. Of them, naturally occurring polyphenols are receiving more attention [16-18].One of the most acknowledged natural flavonoids in liver disease treatment is silymarin. Silymarin is a polyphenolic flavanoid isolated from fruits and seeds of the milk thistle (Silybum marianum) and has been used clinically for many years in Europe and Asia as an anti-hepatotoxic agent for many liver diseases [19]. Despite proved cell protective effects on normal cells, silymarin also possesses cancer-preventive and anti-carcinogenic effects [20-23]. In this

way, apoptotic effect of different concentrations of silymarin in cancer cell lines such as lukemia cells and HepG2 cell lines is published [23-24]. As a matter of fact, silymarin acts as an amphoteric agent on cell proliferation based on the cell type and culture condition.

Several studies have also reported immune-modulatory actions of silymarin. It inhibited iNOS gene expression as well as PGE2 and IL-1β synthesis in LPS-stimulated macrophages [25-26]. At the other hand, increased lymphocyte proliferation, interferon gamma, interleukin (IL)-4 and IL-10 secretions by stimulated lymphocytes in a dose-dependent manner are the other known immune modulatory effects of silymarin [27]. Strikingly, in the mouse model of Con A-induced T cell-dependent hepatitis, silibinin was proven to suppress T cell dependent liver injury, inhibiting intrahepatic expression of tumor necrosis factor, interferon-gamma, IL-4, IL-2, and iNOS [30]. Accordingly, silymarin is a potent immune-response modulator, with both immune-stimulatory and immunosuppressive activities, which may be dependent on its concentration and/or treatment procedure. Despite the inhibitory effect of this agent on different immune factors in various tumor cells, the effect of silymarin on COX-2 and iNOS enzymes level in HepG2 cells remains to be investigated. Thus in the present study, the in vitro effect of silymarin on the HepG2 viability, cellular COX-2 and iNOS levels in the presence of 3 different concentration of silymarin was studied.

Material and Methods

Materials

HepG-2 obtained from National Cell Bank of Iran, Pasteur Institute of Iran (Tehran, Iran). DMEM, FBS, glutamine, antibiotics and trypsin-EDTA solution were obtained from Gibco BioCult (Paisley, UK). MTT powder and Silymarin was purchased from Sigma Chemical Co., St. Louis, MO, USA. COX-2 quantification kit was from Assay designs & Stressgen Inc. (Michigan, USA (BV-K264, San Diego, CA, USA). iNOS quantification kit from Quantakine (R&D Systems Inc, Minneapolis, USA).

Cell culture and treatment

HepG-2 obtained from National Cell Bank of Iran, Pasteur Institute of Iran (Tehran, Iran) was cultured in DMEM medium supplemented with 10% heat inactivated FBS, 100 IU/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine and maintained in a humidified atmosphere with 5% CO_2 at 37°C. The cultured cells were sub-cultured twice each week and the exponentially growing cells were used for all treatments. Silymarin dissolved in DMSO were used for the treatments. 482mM stock of silymarin was employed in this study. At the time of treatment, working solutions were diluted accordingly in DMEM medium. Silymarin were added to the cells, 6 h after the sub-culture. Stock of silymarin was freshly prepared before every treatment. The final concentration of the vehicle (DMSO) never exceeded 0.1%. HepG2 cells exposed t 0.1% DMSO served as controls.

Cell proliferation assay

Proliferation of HepG2 cells was assessed by MTT assay. Cells were plated in 96-well plates at a concentration of 1.5×10^4 cells/well. 24 h after plating, they were washed twice with 500 µl of serum-free medium and were starved by incubation in serum-free medium for an hour at 37 °C. After starvation, cells were treated with silymarin of different concentrations for 24 h. At the end of treatment, media from control and silymarin-treated cells were discarded and 200 µl of MTT containing DMEM (0.5 mg/ml) was added to each well. Cells were then incubated for 4 h at 37 °C in a ۵٪ CO_2 incubator. MTT-containing medium was then discarded and the cells were washed with 1× PBS. Crystals were then dissolved by adding 200 µl of solubilization solution and this was mixed effectively by pipetting up and down. Spectro-photometrical absorbance of the purple blue formazan dye was measured using a microplate reader at 540 nm. Optical density of each sample was then compared with control optical density and graphs were plotted. Based on MTT assay, we selected doses 50 and 75 and 100 µg/ml silymarin treatment for 12 and 24 h in further studies because these are doses below the IC50 (50% inhibitory concentration) value of silymarin in HepG2.

Cell viability estimation by Trypan blue exclusion method

The HepG2 cells in exponential growth phase (2×10^5 cells / cm^2) were transferred to 6- well culture plates. The media was removed and cells were washed with PBS before adding trypsin (500 µl to each well) to detach cells from surface. Plates were then incubated at 37 ºC for 2 min. To stop the trypsin reaction, 500 µl media was added to each well and the contents were transferred to micro tubes and centrifuged at 1200 g (150 rpm) for 5 min. The supernatant was removed and pellet was re-suspended in 1 ml of fresh media, mixed with an equal amount of trypan blue (0.4% in PBS). Then 2 µl of the mixture was placed in a hemocytometer well and the cells were counted under a light microscope (100 X magnification). The number of viable cells (per ml) was counted at different time intervals (12 and 24hr) following cell treatments with 50, 75 and 100 µgr/ml silymarin. The number of cells counted in a set of samples treated with 0.1% DMSO and considered as control. All the assays were carried out in triplicate and cell viability percents presented as mean ± SEM.

Determination of cellular COX-2 level

For this assay HepG2 cells in exponential growth phase (1×10^6 cells) were seeded in 100mm cell culture dish. 6hr after seeding, cells were treated with 50, 75, 100 µg/ml silymarin. A dish containing 0.1% DMSO was served as the control group. In the time intervals of 12 and 24hr, cells were harvested with a rubber policeman and collected cells were centrifuged at1000g for 10min at 4^0C. The supernatant were removed and cell pellet were homogenized in cold buffer (50mM tris-HCl, pH 7.5, 5mM EDTA, 1mM DTT). After that, cells were centrifuged at 10,000 g for 15min at 4^0C and supernatant were removed then COX-2 level in prepared cell lysate were measured using EIA method (COX-2 EIA kit, Assay designs & Stressgen Inc. Michigan, USA) according the kit insert. Optical density was read in 450nm. The assay sensitivity was 0.25ng/ml and coefficient of variation of intra assay was 4.5%. All the assays were carried out in triplicate and presented as Mean ± SD.

Determination of cellular iNOS level

Cellular iNOS level was measured in cells lysate 12 and 24hr after treatments. To prepare cell lysate, cells were harvested using rubber policemen on 12 and 24 hr time intervals. Collected cells were centrifuged at 300g for 5min at 40C. The supernatant were removed and cell pellet were washes twice in sterile PBS. After each wash, cells were centrifuged at 300 x g for 5 minutes and the supernatant was poured off. In the next step, cells were lysis for 10 min at 2-8ºC with cell lysate buffer (1ml buffer per 1×10^6 cells) supplied by the kit. Supernate Homogenized in cold buffer (50mM tris-HCl, pH 7.5, 5mM EDTA, 1mM DTT). iNOS level in each group cell lysate was determined using Quantakine iNOS ELISA kit, (R&D Systems Inc, Minneapolis, USA) based on ELISA technique according to the company's instruction. The assay sensitivity was 0.15U/ml and coefficient of variation of intra assay was 5.2%. All the assays were carried out in triplicate and presented as Mean ± SD.

Statistical analysis

Statistical data were analyzed using SPSS version 15 and student t-test. Significant levels for differences were considered at $P < 0.05$. The percentages of cell proliferation and cell viability are presented as mean ± SEM. The results of biochemical data are presented as mean ± SD. All results are reported as percentage.

Results

Silymarin treatment for 24 h dose-dependently inhibited population growth of HepG2 cells

Silymarin treatment for 24 h dose-dependently inhibited population growth of HepG2 cells Figure 1 shows the effect of silymarin at various doses (0, 6.25, 12.5, 25, 50, 75,100 and 200 µg/ml) on HepG2 cells for 24 h by MTT assay. At the presence of >50µg/ml silymarin, population growth of HepG2 cells were inhibited. Growth inhibition was directly proportional to dose ($p<0.05$). Based on this study, 50 and 75 and 100 µg/ml silymarin lead to 15%, 30% and 49% growth inhibition respectively, and were selected for further procedures.

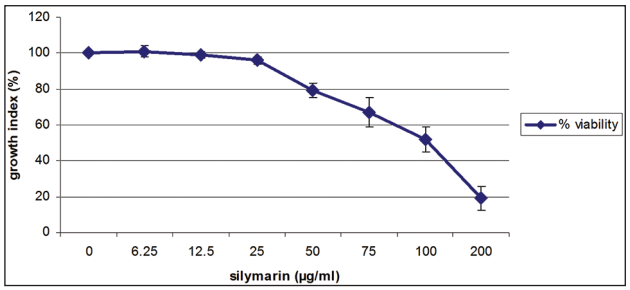

Figure 1. Growth index by MTT method- HepG2 cell growth was not significantly affected by >50µg/ml silymarin, but at concentrations beyond 50 µg/ml silymarin cell proliferation was significantly decrease dose-dependently. IC50 for silymarin was approximately µg/ml.

Silymarin treatment for 12 and 24 h dose-dependently inhibited viability of HepG2 cells

The inhibitory effect of 50, 75 and 100 µg/ml silymarin treatment on HepG2 cells viability is summarized in figure 2. As it is shown, silymarin decrease cell viability time and does dependently in 12 and 24hr after treatment ($p<0.05$). Cell viability in the presence of 50, 75 and 100µgr/ml silymarin was 93%, 83% and 70% in 12hr treated cells and decrease to 77%, 67% and 41% after 24hr treatment.

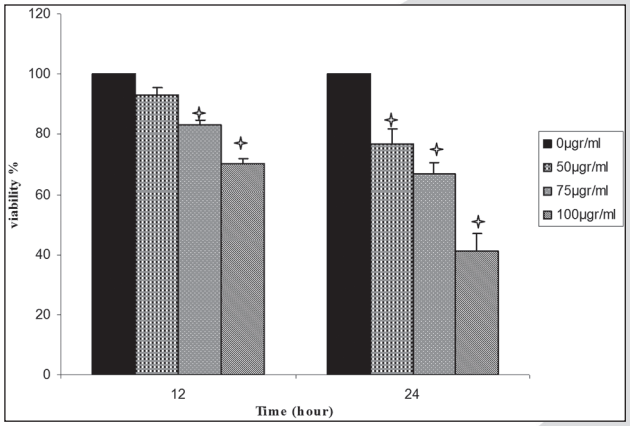

Figure 2. Cell viability percentage in silymarin treated cells vs. control group in 12 and 24 hr time intervals. The decrease in cell viability was significantly lower in the presence of 75 and 100 µg/ml silymarin after 12 hour. In 24 hr interval, cell viability decrease significantly in all silymarin treated cells. ( ) shows significant differences in treated cells ($p<0.05$).

Silymarin treatment for 24 h dose-dependently inhibited COX-2 level of HepG2 cells more than 12hr treatments

After 24hr treatments, COX-2 level in the presence of 50, 75 and 100 µgr/ml silymarin showed significant decrease compared to control group and was 88%, 86% and 71% respectively ($p<0.05$). As it is shown in figure 3, measured COX-2 level in 24hr treated cells were significantly lower compared to respective doses in 12hr time interval($p<0.05$). The level of COX-2 in the presence of 50, 75 and 100 µg/ml silymarin was 50%, 43% and 34% compared to control group.

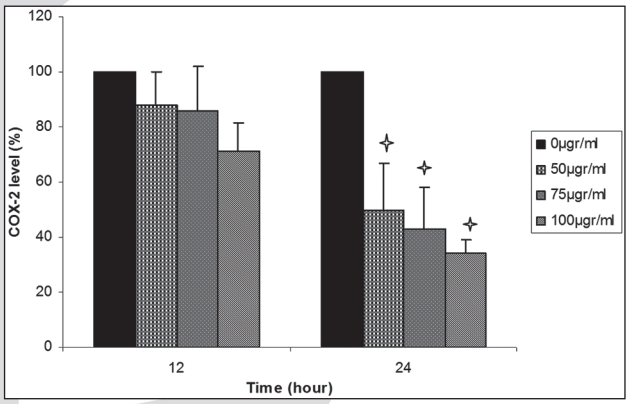

Figure 3. COX-2 level percentage in 12 and 24 hr silymarin treated cells vs. control group- COX-2 level was significantly lower in 24hr treated cells compared to control group. ( ) shows significant differences in treated cells ($p<0.05$).

Silymarin treatments for 24 h dose-dependently inhibited iNOS level of HepG2 cells more than 12hr treatments

Measured iNOS level, in treated groups after 12 hours was 87%, 76% and 71% in 50, 75 and 100µgr/ml silymarin treated cells compared to control group which was not statistically significant. iNOS levels in 24hr treated cells showed significant decrease in treated HepG2 cells in the presence of 75 and 100µg/ml silymarin and reach to 74%, 52% and 40% for 50, 75 and 100 µg/ml silymarin treated cells respectively. The significant decrease occurs in 75 and 100µg/ml treated cells after 24 hr treatments ($p<0.05$) (figure 4).

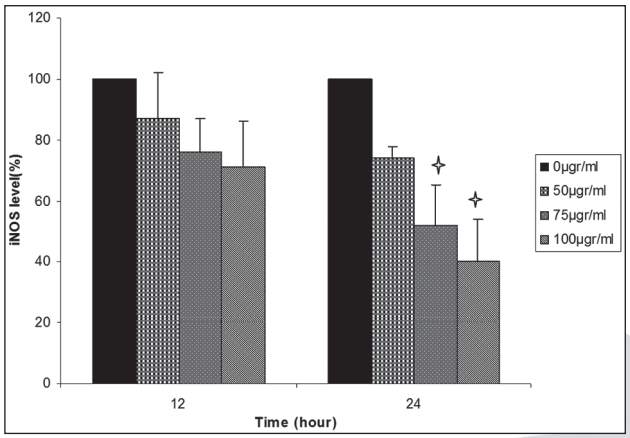

Figure 4. iNOS level percentage in 12 and 24 hr silymarin treated cells vs. control group- enzyme level only showed significant decrease 24hr after 75 and 100 µg/ml silymarin treatments. ( ) shows significant differences in treated cells ($p<0.05$).

Discussion

HepG2 cell viability as well as inflammatory enzymes, COX 2 and iNOS, level were measured 12 and 24 h after 50, 75 and 100 µgr/ml silymarin treatment and compared to untreated HepG2 cell lines. Our results showed that silymarin in a dose dependant manner causes decrease in cell viability. In this study, decrease in COX-2 and iNOS levels was only observed after 24 hour treatment.

Measuring HepG2 cell viability showed that silymarin can increase cell death in a dose dependant manner which was in contrast to our previous result on hbMSCs. In the case of hbMSCs, cell viability in the presence of same concentrations of silymarin (50, 75 and 100µg/ml) increase dose dependently 2 days after treatment [20]. However our results about cell viability was as same as Ramakrishnan et al which reported approximately 10,30 and 50% decreases in HepG2 cell viability in the presence of 50, 75 and 100µgr/ml silymarin, respectively [23].

As shown in fig3 silymarin significantly decrease COX-2 levels 24h after treatment dose dependently. COX-2, a rate-limiting enzyme in the pathway of PG synthesis, is one of the interesting cellular factors and has been suggested to be associated with carcinogenesis [6-12]. In HCC, the expression pattern of COX-2 protein is well correlated with the differentiation grade, suggesting that abnormal COX-2 expression plays an important role in hepato-carcinogenesis [12]. In another study, Kang et al in 2004, showed that >50µg/ml silymarin treatment inhibit cellular COX-2 level in LPS-stimulated macrophage [26].

On the other hand, 75 and 100µg/ml silymarin also decrease iNOS level in HepG2 cells 24hours after treatments. iNOS is one of three key enzymes generating NO from the amino acid L-arginine [15]. In a variety of human malignant tumors, e.g. breast, lung, prostate, bladder, colorectal cancer, malignant melanoma, as well as HCC, expression of iNOS can be observed [15, 25]. Inhibitory effect of >50µg/ml silymarin on iNOS gene expression in LPS stimulated macrophages has been reported by Kang et al in 2002 [25]. In another study, Momeny et al showed that 25, 50 and 75µM silbinin (one of the active fraction of silymarin) can decrease NO production by 16%, 17% and 23%, respectively.

In this study, we also examine the correlation between COX-2 and/ or iNOS level and cell viability in the experiment time intervals. Our finding shows no correlation between inhibition of COX-2 and/or iNOS and cell viability. However, Bae et al in 2001 have proved that COX-2 could promote HCC cell growth and selective COX-2 inhibitor celecoxib could mediate cell apoptosis in HepG2 and Hep3B cell lines [12]. Considering the inhibitory effect of silymarin on HepG2 cell viability, observed decrease in COX-2 and iNOS levels of cells may be due to decreasing the cell number in the presence of silymarin however there was no significant correlation between COX-2 and/or iNOS level and viability percentage. Molecular studies have demonstrated that silymarin toxicity to cancer cells involves the EGFR, insulin-like growth factor-1 receptor, and NF-κB pathways [26-29].

Furthermore, COX2 and iNOS are both inducible enzymes. Gene expression and subsequent mRNA translation for these two enzymes are controlled by various agonists, including NF-kB [15, 1]. As it was reported previously, silymarin is a potent inhibitor for this transcription factor in the hepatoma cell line HepG2 [2]. Thus it can be implied that silymarin can decrease these two enzymes as a NF-kappa B inhibitor.

In conclusion, our result showed a decrease in COX-2 and iNOS level after 24hr treatment with 50, 75 and 100µg/ml silymarin. Based on the existing data about decreasing HepG2 cell proliferation in the presence of silymarin, future studies

should be done to indicated whether silymarin decrease cellular COX-2 and iNOS level due to inducing cell death or inhibition their expression.

References

1. *Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature, 2004; 431: 461-466*

2. *Saliou C, Rihn B, Cillard J, Okamoto T, Packer L. Selective inhibition of NF-UB activation by the flavonoid hepatoprotector silymarin in HepG2. FEBS Letters,1988; 440: 8-12*

3. *Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol, 2005; 5: 749-759*

4. *Blanco M, Rodríguez-Yáñez M, Sobrino T, Leira R, Castillo J. Platelets, inflammation, and athero thrombotic neurovascular disease: the role of endothelial dysfunction. Cerebrovasc Dis, 2005; 20: S32–S39*

5. *Kiritoshi S, Nishikawa T, Sonoda K, Kukidome D, Senokuchi T, Matsuo T. Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy. Diabetes, 2003; 52: 2570–77*

6. *Gupta S, Srivastava M, Ahmad N, Bostwick DG, Mukhtar H. Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. Prostate, 2000; 42: 73–78*

7. *Uefuji K, Ichikura T, Mochizuki H, Shinomiya N. Expression of cyclooxygenase-2 protein in gastric adenocarcinoma. J.Surg.Oncol, 1998; 69: 168–172*

8. *Lim HY, Joo HJ, Choi JH, Yi JW, Yang MS, Cho DY. Increased expression of cyclooxygenase-2 protein in human gastric carcinoma.Clin.Cancer Res, 2000; 6: 519–525*

9. *Yipschneider MT, Barnard DS, Billings SD, Cheng L. Heilman DK, Lin A. Cyclooxygenase-2 expression in human pancreatic adenocarcinomas. Carcinogenesis (Lond.), 2000; 21: 139–146*

10. *Ochiai M, Oguri T, Isobe T, Ishioka S, Yamakido M. Cyclooxygenase-2(COX-2) mRNA expression levels in normal lung tissues and non-small cell lung cancers. Jpn.J.Cancer Res, 1999; 90: 1338–43*

11. *Han G, Boyle JO, Yang, EK, Zhang F, Sacks PG, Shah JP. Cyclooxygenase-2(COX-2) expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res, 1999; 59: 991–994*

12. *Bae SH, Jung ES, Park YM, Kim BS, Kim BK, Kim DG, Ryu WS. Expression of cyclooxygenase-2(COX-2) in hepatocellular carcinoma and growth inhibition of hepatoma cell Lines by a COX-2 inhibitor. Clinical Cancer Research, 2001; 7(5): 1410-18*

13. *Hong SK, Gul YA, Ithnin H, Talib A, Seow HF. Expression of beta-catenin, COX-2 and iNOS in colorectal cancer: relevance of COX-2 adn iNOS inhibitors for treatment in Malaysia. Asian J Surg, 2004; 27(1): 7-10*

14. *Lin Z, Chen S, Ye C. Nitric oxide synthase expression in human bladder cancer and its relation to angiogenesis. Uro res, 2003; 31: 231-235*

15. *Kronke KD, Fehsel K, Bachofen VK. inducible nitric oxide synthase in human disease. Clin Exp Immunol, 1998; 113: 147-156*

16. *Perchelle JP, Perchellet EM. Antioxidants and multi stage carcinogenesis in mouse skin. Free Radic Biol Med, 1989; 7: 377-408*

17. *Dragsted LO. Natural antioxidants in chemoprevention. Arch Toxicol Suppl, 1998; 20: 209-226*

18. *Zhao J, Sharma Y, Agarwal R. Significant Inhibition by the flavonoid antioxidant Silymarin against 12-O-Tetradecanoylphorbol13-Acetate-caused modulation of antioxidant and inflammator Enzymes, and Cyclooxygenase2 and Interleukin-1a Expressionin SENCAR Mouse Epidermis: Implications in the Prevention of Stage I Tumor Promotion. Molecul carcinogen, 1999; S26: 321-333*

19. *FerenciP, DragosicsB, Dittrich H. Randomized Controlled trial of silymarin treatment in patients with cirrhosis of the liver. J Hepatol, 1989; 9: 105-113*

20. *Ahmadi Ashtiani HR, Allameh A, Hamidipour N, Rastegar H, Soleimani M. Increasing in proliferation rate of human bone marrow mesenchymal stem cell in the presence of silymarin. Medical Plants J. article in press, 2010.*

21. *Katiyar SK, Korman NJ, Mukhtar H, Agarwal R. Protective effects of silymarin against photocarcinogenesis in a mouse skin model. J.Natl. Cancer Inst, 1997; 89: 556*

22. *Momeny M, Khorramizadeh MR, Ghaffari SH, Yousefi M, Yekaninejad MS, Esmaeili R. Effects of silibinin on cell growth and invasive properties of a human hepatocellular carcinoma cell line, HepG-2, through inhibition of extracellular signal-regulate kinase1/2 phosphorylation. Euro J Pharmacol, 2008; 591: 13–20*

23. *Ramakrishnan G, Muzio LL, Elinos-Báez CM, Jagan S, Augustine TA, Kamaraj S. Silymarin inhibited proliferation and induced apoptosis in hepatic cancer cells. Cell Prolif, 2009; 42: 229–240*

24. *Gharagozloo M, Amirghofran Z. Effects of silymarin on the spontaneous proliferation and cell cycle of human peripheral blood leukemia T cells. J. Cancer Res. Clin. Oncol, 2007; 133: 525 – 32*

25. *Kang JS, Hwan YJ, Kim MJ, Han SH, Yang KH. Inhibition of Inducible Nitric-Oxide Synthase Expression by Silymarin in Lipopolysaccharide-Stimulated Macrophages. JPET, 2002; 302: 138–144*

26. *Kang JS, Jeon YJ, Park SK, Yang KH, Kim HM. Protection against lipopolysaccharide-induced sepsis and inhibition of interleukin-1b and prostaglandin E2 synthesis by silymarin. Biochem Pharmacol, 2004; 67: 175–181*

27. *Wilasrusmee C, Kittur S, Shah G, Siddiqui J, Bruch D, Wilasrusmee S, Kittur DS. Immunostimulatory effect of Silybum marianum (milk thistle) extract. Med Sci Monit. 2002; 8: BR439–BR443*

28. *Schumann J, Prockl J, Kiemer AK, Vollmar AM, Bang R, Tiegs G. Silibinin protects mice from T cell-dependent liver injury.J Hepatol, 2003; 39:333–340*

29. *Singh RP, Agarwal R. Prostate cancer chemoprevention by silibinin: bench to bedside. Mol Carcinog, 2006; 45: 436–442*

30. *Singh RP, Dhanalakshmi S, Mohan S, Agarwal C, Agarwal R. Silibinin inhibits UVB- and epidermal growth factor-induced mitogenic and cell survival signaling involving activator protein- and nuclear factor-kappaB in mouse epidermal JB6 cells. Mol Cancer Ther, 2006; 5: 1145–1153*

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The use of panoramic radiographs to detect bone loss in rheumatic patients

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Abstract

Objectives: Panoramic radiographic-based indices and other conventional radiography techniques have been used to detect osteoporosis. The aim of this study was to determine whether it was possible to predict the generalized bone losses of patients with rheumatoid disease (RA /AS) using panoramic radiography.

Materials and Methods: Data of 74 patients were obtained from the archives of the rheumatology and dental clinics at Gaziantep University. This data included dual energy X-ray absorptiometry (DEXA) values, panoramic radiography results, rheumatoid status, age, gender, height, weight, and body mass index (BMI). The morphology of the mandibular inferior cortex was determined and classified according to Klemetti's classification.

Results: An inter-observer agreement with a kappa value of substantial agreement (κ: 0.677) was found between the panoramic radiograph assessments of the Mandibular Cortical Index (MCI) by three independent examiners. Coefficients between the BMD of the lumbar spine and femoral neck were substantial (κ: 0.609) and moderate (κ: 0.471), respectively. The sensitivity, specificity, PPV, and NPV were determined 46, 86.7, 80.4, and 57.3 for femoral T score and 69.6, 85.2, 66.7, and 86.8 for lumbar T score, respectively.

Conclusions: Dental panoramic radiography may be considered as a predictor of bone loss in RA/AS patients within the limitations of the present study.

Key words: Rheumatoid arthritis, Panoramic Radiography, Osteoporosis, Mandibular Bone Loss,

Introduction

Epidemiological studies have clearly demonstrated that patients diagnosed with rheumatoid arthritis (RA), systemic lupus erythematosus, or ankylosing spondylitis (AS) have accelerated systemic bone loss leading to an increased risk of bone fragility and sometimes fractures[1]. Rheumatoid arthritis is an autoimmune and chronic destructive joint disease that also affects several organs and systems[2] Functional disability is an inevitable result when patients are improperly treated or not treated at all. In the pathogenesis, many pro-inflammatory cytokines orchestrate matrix metalloproteinases, cathepsins, and osteoclasts that result in bone erosion[3-6]. Bone loss in RA patients results in focal articular bone erosion, periarticular bone loss, and generalized osteopenia[7] and is associated with increased risk of hip and vertebral fractures[8]. Risk factors include age, body mass index (BMI), menopausal status, reduced morbidity, disease activity, the influence of anti-rheumatic therapy, especially corticosteroids, and disease duration[7].

Ankylosing spondylitis is also a chronic inflammatory disease that predominantly affects young men. Inflammatory enthesopathy progressing to ossification and ankylosis is the pathologic basis of the disease. Diffuse osteoporosis responsible for loss of bone strength is a feature of AS. The bone loss predominates at the spine and vertebral fractures could be a complication in the ongoing disease period[9].

Bone fragility is dependent on several inter-related parameters, such as bone mass, bone structural properties such as geometry and microarchitecture and material properties of the bone tissue. In order to predict the risk of fracture, bone mass or bone mineral density (BMD) is measured using several techniques, including single or dual photon absorptiometry, quantitative computed tomography single or dual X-ray absorptiometry and quantitative ultrasound[10-12]. The effect of osteoporosis on the jaws and on residual ridge resorption has been studied ex-

tensively using new radiographic methods. Most of these methods are expensive and are often unavailable to practitioners.

Panoramic radiography is often used by dentists. Attempts have been made to demonstrate changes in jaw bones caused by osteoporosis using panoramic images[13]. Recent studies have indicated that a dental panoramic radiograph may be one of the most useful tools to detect low BMD, high bone turnover, or high risk of osteoporotic fracture in women[13-18]. Several indices have been put forward to detect osteoporosis using panoramic radiography, including the Panoramic Mandibular Index, Mandibular Cortical Index (MCI), Antegonial Index, Gonial Index, Mandibular Cortical Width, and Mental Index[13, 14, 17, 19, 20]. The literature does not contain any information about determining the generalized bone loss of patients with rheumatic diseases using panoramic radiography. The aim of this study is to determine whether it is possible to reveal the generalized bone loss of patients with RA and AS using panoramic radiography and if the panoramic osteoporotic indexes are correlated well with systemic osteoporosis in rheumatic diseases.

Materials and methods

Subjects

Seventy four patients from the archives of Gaziantep University School of Dentistry who had rheumatic diseases of rheumatoid arthritis (RA) or ankylosing spondylitis (AS) in their anamnesis, had panoramic mandibular radiographs available and data regarding dual energy x-ray absortiometry (DEXA) values, disease status, age, gender, height, weight, and body mass index (BMI) were available from the archives of the rheumatology clinic were included retrospectively. The BMI equals subjects weight (kg) divided by the height (m) squared (BMI; kg/m2). The time and causes of tooth loss were unknown. The study was approved by the Local Ethics Committee.

Vertebral and Femoral BMD

The BMD values for the femoral neck and lumbar spine (L1-L4) were determined using a DEXA apparatus (Lunar DPX, Lunar Corporation, WI, USA). Height, weight, BMI, and T-scores were also determined in this way. Each patient was classified into one of three groups according to World Health Organization (WHO) criteria[21-24]: normal (T-score >-1.0), osteopenic (T-score of -1 to -2.5), or osteoporotic (T-score of <-2.5) at the skeletal site on the basis of the BMD data. Overall, individual patients were also classified as having low skeletal BMD if they were osteopenic or osteoporotic at any one or more sites and the others were classified as normal.

Panoramic Radiographic Assessment

Dental digital panoramic radiographs were taken for each patient using the same X-ray machine (Orthophos XG5 DS / Ceph, Sirona Dental System, Bensheim, Germany) and were evaluated using a personal computer running the Windows XP operating system and Sidexis XG software version 2.3 (Sirona Dental Systems, Bensheim, Germany). The radiographs were evaluated by experienced observers in Oral and Maxillofacial Radiology (Observer 1) and Periodontology (Observers 2 and 3). Original images were altered TIFF format prior to assessment and evaluated on the dark room. The morphology of the mandibular inferior cortex was determined by a bilateral observation of the distal side of the mental foramen of the mandible using Klemetti's three-graded classification, as follows (Figure 1):

- Class I, the endosteal margin of the inferior cortex is smooth;
- Class II, the endosteal margin shows semilunar defects (lacunar resoption) with the formation of endosteal cortical residues and one to three layers are thick; and
- Class III, the cortex is obviously porous with dense endosteal residues.

The MCI classification was performed by three independent observers and at the end of the study, it was re-examined independently by the three observers and consensus was achieved.

Statistical Analysis

Student t and ANOVA tests were used for comparisons and the relationship between categorical variables was investigated using chi-square analysis. Kappa statistics were used to determine interobserver agreement. The sensitivity, specificity, and positive and negative predictive values of the

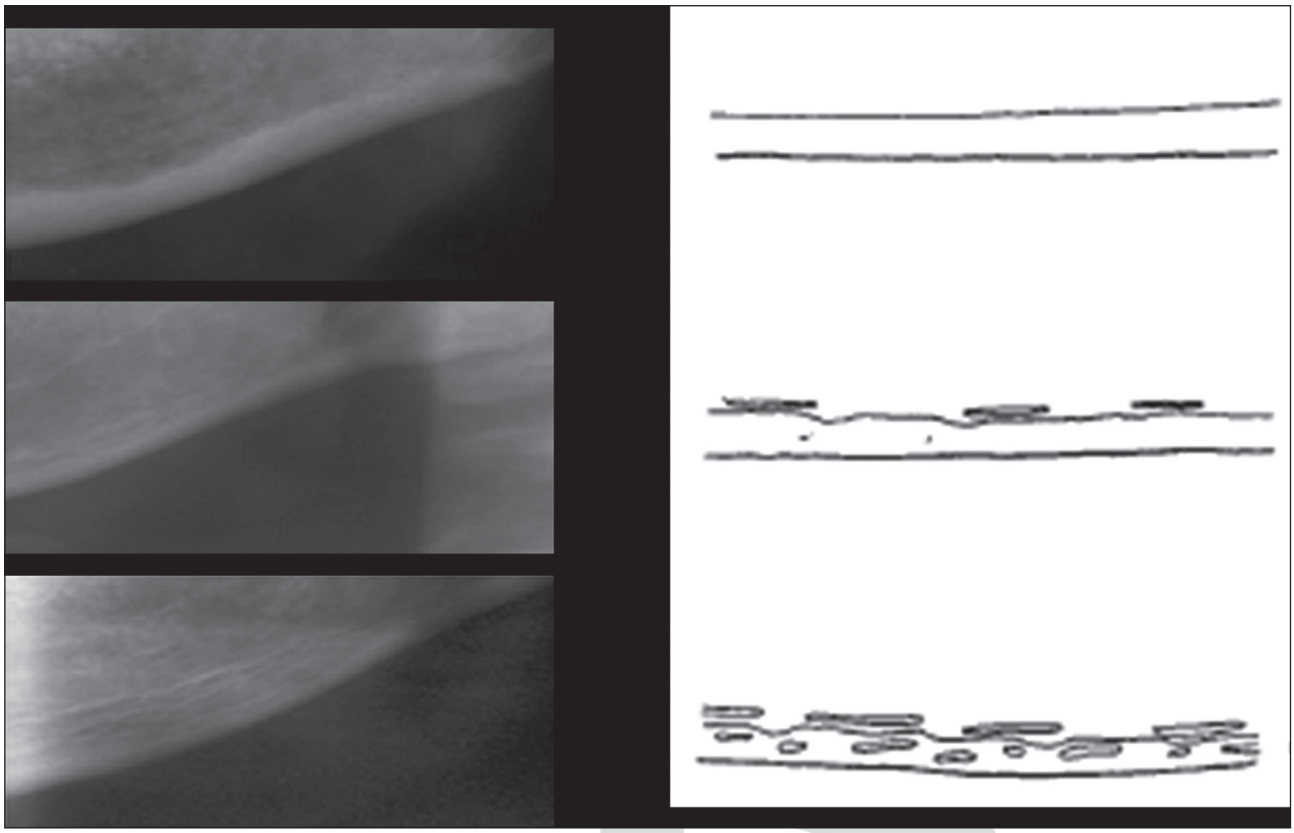

Figure 1. Classification of mandibular cortical index with schematic and radiographic projection

mandibular variables in diagnosing bone loss were tested. Analyses were performed using version 11.5 of the SPSS statistics program for Windows and R package version 2.10.1 (SPSS version 11.0, SPSS Inc., Chicago, IL, USA); P values lower than 0.05 were accepted as significant.

Results

Seventy four patients were included in the study (21 males and 53 females with a 1/3 ratio) 43 of them had RA and 31 had AS. Out of the 74 patients, 56 of them were dentulous, 10 of them were partially dentulous and 8 were edentulous. Among the 10 partialy dentulous patients, 6 of them were unilateral, 4 were bilateral partially dentulous. The patient demographics according to to lumbar spine and femoral neck DEXA scores are shown in Table 1.

Based on the dental panoramic radiography assessed by the three examiners, the sensitivity, specificity, predictive values of the patients with reduced skeletal bone density, and T-scores of the lumbar spine and femoral neck were considered and are presented in Tables 2 and 3. 47 of the 52 subjects with osteopenia or osteoporosis were identified (specificity 85%) while 17 of the 22 subjects with normal lumbar T-scores were identified (sensitivity 70%). However, depending on the femoral neck scores, 40 individuals with normal values were identified (specificity 87%) and of the remaining 34 with femoral osteopenia or osteoporosis, half of them were predicted (sensitivity 46%). When the T-score of the femoral neck was used as a gold standard, the specificity was nearly twice as high as the sensitivity.

Inter-observer agreement among the three independent examiners was reached regarding the assessment of the MCI based on panoramic radiographs, with a substantial kappa value of 0.677. Coefficients between three observers and the T scores of the lumbar spine and femoral neck were substantial (κ: 0.609) and moderate (κ: 0.471), respectively. Agreement between the three observers was reached when subjects were classified as normal (C1) and diseased (combined C2 and C3), with a moderate kappa value of κ: 0.556. Coefficients between the T-scores of the lumbar spine and femoral neck were moderate (κ: 0.453) and fair (κ: 0.387), respectively. When the kappa value between T-scores of the lumbar spine and femoral neck were assessed, there was fair agreement (κ: 0.388).

Table 1. Descriptive statistics of the subjects according to Lumbar and Femoral T score

		N	RA	AS	Age	BMD	T score	BMI
Lumbar Spine	Normal	22	9	13	40.7 ± 8.5	1.0 ± 0.1	-0.3 ± .9	30.1 ± 5.3
	Osteopenia	33	20	13	45.3 ± 12.2	0.9 ± .01	-1.6 ± .6	28.8 ± 8.6
	Osteoporosis	19	14	5	50.9 ± 11.5	0.7 ± 0.1	-2.5 ± 1.2	27.0 ± 5.8
Femural Neck	Normal	40	22	19	43.4 ± 10.5	0.9 ± 0.1	-0.9 ± 1.2	29.9 ± 5.3
	Osteopenia	22	14	8	46.5 ± 11.6	0.9 ± 0.1	-1.4 ± 0.8	28.7 ± 9.5
	Osteoporosis	12	7	4	50.1 ± 13.7	0.7 ± 0.1	-2.7 ± .8	24.8 ± 6.2

N: Number of patients, RA: Rheumatoid Artritis, AS: Ankylosing Spondilitis, BMD: Bone Mineral Density, BMI: Body Mass Index

Table 2. Observer variability of the BMD value using DEXA

Observers	Sensitivity % (95%CI)	Specificity % (95%CI)	LR+	LR-	PPV %	NPV %
Observer 1	47.6 (32.0- 63.6)	85.7 (69.7-95.2)	3.3 (1.4-7.9)	0.6 (0.4-0.8)	80.0 (59.3-93.2)	57.7 (43.2-71.3)
Observer 2	50.0 (34.2- 65.8)	88.6 (73.2-96.8)	4.4 (1.7-11.6)	0.6 (0.4-0.8)	84.0 (63.9-95.5)	59.6 (45.1- 72.9)
Observer 3	40.5 (25.6- 56.7)	85.7 (69.7-95.2)	2.8 (1.2-6.9)	0.7 0.5-0.9)	77.3 (54.6-92.2)	54.5 (40.6-68.0)
Grand Mean	46.0	86.7	3.5	0.6	80.4	57.3

PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR+: Positive Likelihood Ratio, LR-: Negative Likelihood Ratio

Table 3. Relationship between MCI and femoral and lumbar T-score

Observers	Sensitivity % (95%CI)	Specificity % (95%CI)	LR+	LR-	PPV %	NPV %
Observer 1	73.9 (51.6-89.8)	85.2 (72.9-93.4)	4.9 (2.5- 9.9)	0.3 (0.2-0.6)	68.0 (46.5-85.1)	88.5 (76.6-95.7)
Observer 2	69.6 (47.1-86.8)	83.3 (70.7-92.1)	4.2 (2.2-8.0)	0.4 (0.2- 0.7)	64.0 (42.5-82.0)	86.5 (74.2-94.4)
Observer 3	65.2 (42.7- 83.6)	87.0 (75.1-94.6)	5.0 (2.4-10.7)	0.4 (0.2-0.7)	68.2 (45.1-86.1)	85.5 (73.3-93.5)
Grand Mean	69.6	85.2	4.7	0.4	66.7	86.8

PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR+: Positive Likelihood Ratio, LR-: Negative Likelihood Ratio

Discussion

In the present study, the aim was to show the validity of the MCI in the diagnosis of reduced skeletal BMD for patients with RA or AS. Data on the sensitivity and specificity of mandibular cortex measurement related to a standard T-score assessment of the lumbar spine show 70 % sensitivity and 85 % specificity. However, femoral DEXA show 46 % sensitivity and 87 % specificity for the mandibular cortex measurement. It also demonstrated there was substantial agreement among the three observers and T-score values of the lumbar spine while femoral DEXA and the observers showed moderate agreement. The lumbar spine values indicate that the MCI may be used to determine bone loss in RA and AS patients.

Oral clinical findings for temporomandibular joints (TMJs) affected by RA and AS are similar to those for other joints. Symptoms include pain, swelling, impaired movement, and crepitation[23]. Malocclusion of the teeth and an anterior open bite are also mentioned in the literature[25]. Some recent studies show the relationship between periodontitis and RA[2, 26]. Bone loss of the maxillary or mandibular jaws in RA patients is inevitable. Therefore, MCI index used as a panoramic radiographic prediction for the detection of osteoporosis of the mandible may be indicated for the diagnosis of bone loss in RA and AS patients.

There are a number of studies related to the prediction of bone loss involving panoramic-based indices such as MCI, PMI, GI, MI, MCW, and AI. Of these indices, PMI and MCW, and especially MCI, are the most studied[13, 14, 18, 19, 27]. Although some researchers have concluded that panoramic radiography should not be used to assess patients' status regarding osteoporosis[13], others have sug-

gested that it could be a reliable screening tool for osteoporosis[25, 28]. The MCI was used to diagnose bone loss in the present study because it is one of the most useful indices and has a simple classification for the detection of bone loss. The MCI was first presented by Klemetti *et al*. in 1994 as a new radiomorphometric index of mandibular cortical bone[13]. Most studies related to MCI show it may be useful to identify osteoporosis in clinical dental practices.

To determine BMD both femoral neck and lumbar spine DEXA values are evaluated. There are a number of studies related to the determination of bone loss using panoramic-based indices that primarily used DEXA for comparison[13, 19, 27]. Lee *et al*.[28] and Klemetti *et al*.[27] reported no differences between femoral neck and lumbar T-scores or BMD values. In the present study, MCI values were determined by three observers. There was substantial agreement (κ: 0.677) among the three observers as well as with the lumbar spine DEXA results (κ: 0.609), but moderate agreement with the femoral neck DEXA results (κ: 0.471) when using Klemetti's three-graded classification (normal, osteopenic, and osteoporotic). However, when subjects were classified as normal (C1) and diseased (combined C2 and C3), kappa values and the BMD of the lumbar spine and femoral neck were moderate (κ: 0.453) and fair (κ: 0.387), respectively. Higher kappa values and better agreement were assumed when groups were combined, but the results of the present study show that agreement lessened when a two-graded classification was used.

When the T-score of the femoral neck was used, the low sensitivity indicated that less than half of the patients with low skeletal T-scores could not be identified by the three observers. However, the sensitivity and negative predictive value (NPV) were high when the T-score of the lumbar spine was used. The positive predictive value (PPV) suggests that observers correctly identified 80% of the osteopenic/osteoporotic patients according to the femoral neck T-score; the PPV of the lumbar spine was diagnosed at a lower percentage. In addition, the results of the present study also show that higher positive likelihood ratio (LR+) and lower negative likelihood ratio (LR-) values were found according to the T-score values of the lumbar spine. This indicates that diseased subjects were distinguished more accurately than the patients without osteoporosis.

Some obstacles to the use of panoramic radiography for the detection of bone loss using panoramic-based indices were encountered. These included a lack of sharpness in the panoramic image, an undefined mental foramen, and superimposition of anatomical structures such as hyoid bones on mental and cortical regions of the panoramic image. These all made it difficult to distinguish the endosteal border of the cortex. Therefore, this limits agreement among the observers. Systemic bone loss was encountered in patients with RA and AS and we had no information about whether this definitely affected the mandible of each patient due to his/her rheumatic disease. Having RA and AS are risk factors for osteoporosis independent from the cortisone treatment. Additionally most of the rheumatologists use steroids in lower doses in patients with RA. Our study RA population were also using steroids not more than 5 mg/day prednisolone in their daily practice. It is interestingly nearly impossible to find a RA group whom treated perfectly without steroids and osteoporosis. This may have a small contribution on over all bone loss of the patients the small sample size also limits the interpretation of the present findings. Further investigation of a larger population would be necessary to ensure the reliability of these findings. The number of existing teeth was not included in the present correlation equation for BMD status. Although Kribbs[29] and Daniell[30] have stated that the number of teeth is a useful predictor for vertebral fracture due to osteoporosis, Elders *et al*.[31] and Klemetti *et al*.[13] could not find any association between the number of teeth and the risk of vertebral fracture due to osteoporosis. This matter should be investigated in further studies to better understand these contradictions.

In conclusion, the results of the present study demonstrate that dental panoramic radiography may reveal bone loss in RA and AS patients and that panoramic radiographic measurement may be associated with subclinical risk factors for systemic diseases such as RA and AS. Hence, dentists should be alert for complications related to systemic bone lose during dental treatment for patients with rheumatic diseases.

References

1. *Sinigaglia L, Varenna M, Girasole G, Bianchi G. Epidemiology of osteoporosis in rheumatic diseases. Rheum Dis Clin North Am 2006; 32: 631-658.*

2. *Bartold PM, Marshall RI, Haynes DR. Periodontitis and rheumatoid arthritis: a review. J Periodontol 2005; 76: 2066-2074.*

3. *Woolley DE, Crossley MJ, Evanson JM. Collagenase at sites of cartilage erosion in the rheumatoid joint. Arthritis Rheum 1977; 20: 1231-1239.*

4. *Haynes DR, Crotti TN, Loric M, Bain GI, Atkins GJ, Findlay DM. Osteoprotegerin and receptor activator of nuclear factor kappaB ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. Rheumatology (Oxford) 2001; 40: 623-630.*

5. *Woolley DE, Evanson JM. Collagenase and its natural inhibitors in relation to the rheumatoid joint. Connect Tissue Res 1977; 5: 31-35.*

6. *Woolley DE, Evanson JM. Effect of cartilage proteoglycans on human collagenase activities. Biochim Biophys Acta 1977; 497: 144-150.*

7. *Horwood N. Lymphocyte-derived cytokines in inflammatory arthritis. Autoimmunity 2008; 41: 230-238.*

8. *Walsh NC, Crotti TN, Goldring SR, Gravallese EM. Rheumatic diseases: the effects of inflammation on bone. Immunol Rev 2005; 208: 228-251.*

9. *Senel K, Baykal T, Melikoglu MA, et al. Serum melatonin levels in ankylosing spondilitis: correlation with disease activity. Rheumatol Int; 31: 61-63.*

10. *Kalender WA, Suess C. A new calibration phantom for quantitative computed tomography. Med Phys 1987; 14: 863-866.*

11. *Salamone LM, Krall EA, Harris S, Dawson-Hughes B. Comparison of broadband ultrasound attenuation to single X-ray absorptiometry measurements at the calcaneus in postmenopausal women. Calcif Tissue Int 1994; 54: 87-90.*

12. *Mazess RB, Barden HS. Measurement of bone by dual-photon absorptiometry (DPA) and dual-energy X-ray absorptiometry (DEXA). Ann Chir Gynaecol 1988; 77: 197-203.*

13. *Klemetti E, Kolmakov S, Kroger H. Pantomography in assessment of the osteoporosis risk group. Scand J Dent Res 1994; 102: 68-72.*

14. *Taguchi A, Suei Y, Ohtsuka M, Otani K, Tanimoto K, Ohtaki M. Usefulness of panoramic radiography in the diagnosis of postmenopausal osteoporosis in women. Width and morphology of inferior cortex of the mandible. Dentomaxillofac Radiol 1996; 25: 263-267.*

15. *Taguchi A, Suei Y, Sanada M, et al. Detection of vascular disease risk in women by panoramic radiography. J Dent Res 2003; 82: 838-843.*

16. *Bollen AM, Taguchi A, Hujoel PP, Hollender LG. Case-control study on self-reported osteoporotic fractures and mandibular cortical bone. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90: 518-524.*

17. *Devlin H, Horner K. Mandibular radiomorphometric indices in the diagnosis of reduced skeletal bone mineral density. Osteoporos Int 2002; 13: 373-378.*

18. *Nakamoto T, Taguchi A, Ohtsuka M, et al. Dental panoramic radiograph as a tool to detect postmenopausal women with low bone mineral density: untrained general dental practitioners' diagnostic performance. Osteoporos Int 2003; 14: 659-664.*

19. *Drozdzowska B, Pluskiewicz W, Tarnawska B. Panoramic-based mandibular indices in relation to mandibular bone mineral density and skeletal status assessed by dual energy X-ray absorptiometry and quantitative ultrasound. Dentomaxillofac Radiol 2002; 31: 361-367.*

20. *Gulsahi A, Yuzugullu B, Imirzalioglu P, Genc Y. Assessment of panoramic radiomorphometric indices in Turkish patients of different age groups, gender and dental status. Dentomaxillofac Radiol 2008; 37: 288-292.*

21. *Gough AK, Lilley J, Eyre S, Holder RL, Emery P. Generalised bone loss in patients with early rheumatoid arthritis. Lancet 1994; 344: 23-27.*

22. *Cakur B, Sahin A, Dagistan S, et al. Dental panoramic radiography in the diagnosis of osteoporosis. J Int Med Res 2008; 36: 792-799.*

23. *Scutellari PN, Orzincolo C. Rheumatoid arthritis: sequences. Eur J Radiol 1998; 27 Suppl 1: S31-38.*

24. *Erdogan O, Incki KK, Benlidayi ME, Seydaoglu G, Kelekci S. Dental and radiographic findings as predictors of osteoporosis in postmenopausal women. Geriatr Gerontol Int 2009; 9: 155-164.*

25. *Ishizaki K, Suzuki K, Mito T, Tanaka EM, Sato S. Morphologic, functional, and occlusal characterization of mandibular lateral displacement malocclusion. Am J Orthod Dentofacial Orthop 2010; 137: 454 e451-459; discussion 454-455.*

26. *Kobayashi T, Murasawa A, Komatsu Y, et al. Serum cytokine and periodontal profiles in relation to disease activity of rheumatoid arthritis in Japanese adults. J Periodontol 2010; 81: 650-657.*

27. *Klemetti E, Kolmakov S, Heiskanen P, Vainio P, Lassila V. Panoramic mandibular index and bone mineral densities in postmenopausal women. Oral Surg Oral Med Oral Pathol 1993; 75: 774-779.*

28. *Lee K, Taguchi A, Ishii K, et al. Visual assessment of the mandibular cortex on panoramic radiographs to identify postmenopausal women with low bone mineral densities. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100: 226-231.*

29. *Kribbs PJ. Comparison of mandibular bone in normal and osteoporotic women. J Prosthet Dent 1990; 63: 218-222.*

30. *Daniell HW. Postmenopausal tooth loss. Contributions to edentulism by osteoporosis and cigarette smoking. Arch Intern Med 1983; 143: 1678-1682.*

31. *Elders PJ, Habets LL, Netelenbos JC, van der Linden LW, van der Stelt PF. The relation between periodontitis and systemic bone mass in women between 46 and 55 years of age. J Clin Periodontol 1992; 19: 492-496.*

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Aerobic exercises relieve symptoms of primary chronic insomnia

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Abstract

Purpose: The aim of the study was to evaluate the effect of moderate aerobic exercises on symptoms of chronic primary insomnia.

Participants: Twenty insomniac patients were included (age=35±5.42 yrs) and were divided into two equal groups (study and control).

Methods: Sleep duration and daytime symptoms were assessed before and after treatment by using a sleep diary and the Epworth Sleepiness Scale (ESS) respectively. Both groups received cognitive/behavioral and drug therapy. Additionally, patients of the study group were enrolled in a supervised structured moderate aerobic exercise program (30 min, three times/week for four weeks).

Results: The findings revealed significant increase in both the total number of sleep hours and ESS mean values in the study group compared to those of the control one after treatment (p=0.0001). Moreover, there was significant increase in the number of sleep hours in the study group at the fourth week compared to that at the second week of the intervention (p=0.02).

Conclusion: Moderate aerobic exercise was effective in improving symptoms of chronic primary insomnia and may be prescribed as an alternative treatment to drugs in the future.

Key words: Aerobic exercises, Sleep diary, Epworth Sleepiness Scale, Insomnia, Sleep disorders.

Introduction

Insomnia-a common symptom caused by medical, behavioral, or psychological conditions-is defined as difficulty falling asleep, staying asleep, or having poor-quality or non-restorative sleep. Primary insomnia, as defined refers to a persistent sleep disturbance which is not connected to a current psychiatric or physical condition. Chronic insomnia occurs for at least three nights a week for one month or more. It is estimated to affect 10–19% of all individuals[1-4]. Insomnia interrupts the natural sleep cycle, which can be hard to restore. Sufferers typically complain of being unable to keep their eyes closed for more than few minutes at a time, or of tossing and turning in bed. Some insomniacs unwittingly perpetuate their complaint by napping in the late afternoon or early evening, leading to wakefulness at bedtime and more insomnia. Chronic insomnia may cause extensive sleep deprivation with deleterious effects to physical and mental health[5]. Consequences include mood disturbances, medication habituation, memory impairment, daytime fatigue, vocational and interpersonal difficulties, increased health care utilization, impaired health status[6-8]. Notwithstanding the variety of behavioral and medical options to treat insomnia, treatments are ultimately inefficient. In the current study, it was hypothesized that aerobic exercise-known to positively affect sleep quality-might be used as an efficacious alternative to drug therapy.

Participants and Methods

Participants

Twenty consecutive patients (19 females and one male) were selected from King Abdul-Aziz University Hospital and King Abdul-Aziz Medical City in Jeddah, Kingdom of Saudi Arabia. The duration of symptoms ranged from 1–4 years.

Inclusion criteria: Age ranged from 35–45 yrs and insomnia was primary and chronic (> one month).

Exclusion criteria: Pregnant and/or lactating women; restless leg syndrome, and subjects who were unable to be enrolled in the exercise program.

Written informed consent was obtained from subjects prior to the start of the study. Patients were randomly divided into two equal groups: study and control.

Methods

Assessment

Clinical history and daytime sleepiness

All patients were personally interviewed before and after treatment at the same daytime and rated according to Epworth Sleepiness Score (ESS)[9]. A score of 0–6 was suggestive of insomnia[10].

Sleep diary

Patients were given clear verbal instructions on how to use the sleep diary on the first day of their recruitment. The sleep diary is a useful tool to diagnose sleep disorders and to monitor the success of treatment. It is a record of an individual's sleep and waking hours with related information, usually for 4 weeks duration (appendix). It is self-reported or can be recorded by a caregiver[9,11]. In the present study, the sleep diary was filled out by each patient for one week (considered as pre treatment) and then during of the 2nd week and 4th week of intervention (considered as post-treatment).

Treatment

Medical treatment: Sedatives, tranquilizers, or anxiolytics was prescribed at the discretion of a physician specialized in sleep disorders. *Behavioral therapy:* Patients were instructed to follow environmental considerations such as maintaining a quiet, dark, relatively cool, well-ventilated, comfortable sleep environment. Also, avoidance of heavy evening meals, smoking, caffeine, or fluids prior to bedtime in order to reduce the potential effect of gastric discomfort in addition to avoidance of high caloric intake, stimulants, or full bladder. Miscellaneous considerations (sleep hygiene and stimulus control): Participants were also asked to establish regular sleep and waking up hours (including weekends and vacations), use the bed for sleep only (not for other activities such as reading and watching TV), avoid naps or spending excessive time in bed trying to fall sleep, and take a warm bath about 1.5–2 hours before bedtime to help the body to relax. If awake on bed for longer than 20 min, they were instructed to get out of bed, go into another room, and do a quiet activity using dim lighting until they felt sleepy. Patients were also encouraged to write down their thoughts and emotions to allay worries[12]. Deep breathing exercises were also practiced twice daily for 10 min. to promote relaxation and achieve a significant reduction in stress-related symptoms, calm the body, promote sleep, and shorten sleep onset latency[13]. Additionally, subjects were instructed to wear loose fitting cloth, lie down on their back, and stretch out comfortably as a relaxation exercise at bedtime to reduce the symptoms of stress and anxiety. Training involved tensing particular muscle groups for 7–10 sec, followed by release for 15–20 sec. The sequence consisted of clenching the hands into fists, wrinkling the forehead, closing the eyes tightly, pressing the lips tightly, pressing the back of the head against the floor, pressing the buttocks, and clenching the thighs[14].

Treadmill training: In addition to the previously mentioned treatment programs, the study group was enrolled in a supervised treadmill aerobic exercise program. They were asked to walk for 30 min, six hours before bedtime (three days a week for four weeks) on a treadmill (CYBEX, Medway, Model 530T, Z01-20530T9034NN007, AMPS 151-PHASE, USA) in the afternoon or early evening. Each session consisted of (i) A 5-min. warm-up period performed by leaning against the wall, placing most of the weight on the rear foot, and pushing the hip down and forward while keeping the rear leg straight and the heel on the ground for 5–10 sec. (same for the other leg) to stretch the calf muscles. (ii) A 20-min. exercise period at a speed of 4–6 km/h, which was adjusted accordingly to the heart rate (HR) range by determining the maximum HR (220 - age of the patient) and the exercise HR by using the Karvonen formula (HR rest + % of intensity ´ [HRmax - HRrest], in which % of intensity was 60–70%); and finally, (iii) A cool-down period similar to the warm- up period[15].

Statistical analysis

Data were presented as means ± standard deviation. Paired and unpaired *t*-tests, Wilcoxon and Mann–Whitney tests were used to analyze data. *p*-value was <0.05. Data were analyzed using the SPSS software version 10.

Results

Sleep diary

Sleep hours in the study and control groups were significantly different at baseline (20.60 ± 6.24 and 14.05 ± 4.51, respectively, p=0.02) and after treatment (50.85 ± 6.32 and 13.90 ± 4.31, respectively, p=0.0001). The control group exhibited no significant difference between the initial and the final time of the study period (p=0.75). On the other hand, the number of sleep hours in the study group significantly increased after treatment compared to that before treatment (p=0.0001) (Figure 1).

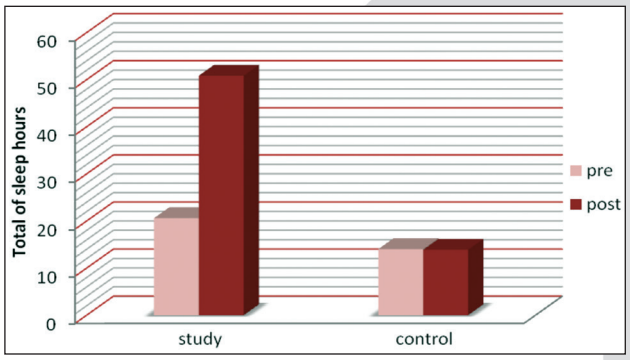

Figure 1. Mean values of total sleep hours before and after treatment in the study and control groups

In addition, in the study group, the number of sleep hours increased significantly by the end of 4th weeks compared to that of the 2nd week, with being 50.85 ± 6.32 and 39.90±6.38 hrs, respectively (p = 0.02) (Figure 2).

Daytime sleepiness

At baseline, no significant difference was found regarding ESS mean values between the study and control groups (p=0.94). However, after treatment, it was significantly higher in the study group (p=0.0001). Additionally, the ESS mean values significantly increased after treatment compared to that before treatment in the study group ((p=0.05) (Table 1).

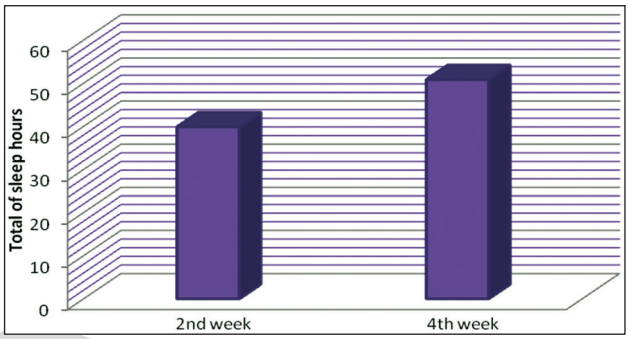

Figure 2. Mean values of total sleep hours by the end of second and fourth week of intervention in the study group

Discussion

Insomnia is a common disorder characterized by insufficient or poor sleep quality associated with adverse daytime consequences in the form of tiredness, fatigue, and potential mood and cognitive impairment. The aim of the present work was to evaluate the influence of aerobic exercises on improving symptoms of insomnia. The outcomes revealed a significant increase in the total sleep hours and improvement in ESS scores. These findings are in accordance with those of Sherrill et al[16] who investigated the influence of moderate exercise on self-reported sleep disorders. They reported that regular exercises might be a useful therapeutic modality in the treatment of sleep disorders. Similarly, Tanaka et al[17] investigated the effect of a four-week exercise program on sleep quality carried out in the evening, and reported significant reduction in the wake time after sleep onset. Mental and physical health was also improved. In comparison with other treatment regimes, a number of adverse side effects of sleeping pills were reported by researchers as cognitive impairment, daytime sedation, motor in-coordination, and risk of motor vehicle accidents, slips and falls. In addition, the effectiveness and safety of such agents remain to be determined and are not recommended for long-term use[18,19]. On the other hand, behavioral/

Table 1. Comparison between study and control groups regarding Epworth Sleepiness Scale (ESS) mean values before and after treatment

	ESS mean values		
	Study group	**Control group**	***p* value**
Before treatment	2.8 ± 0.79	2.8 ± 0.92	0.94
After treatment	12.4 ± 3.09	3.4 ± 0.97	0.0001*
p value	0.05*	0.13	

*The data represent the mean ± standard deviation. *Statistically significant*

cognitive therapy (CBT) has proved effective not only in people with primary insomnia but also in insomnia co morbid with psychiatric and medical illness such as depression, cancer, and chronic pain[20]. But it is costly and difficult to deliver because yet, there is a woeful lack of trained providers and many large metropolitan areas may not have even a single highly-trained CBTspecialist[21]. In contrast, exercise is a healthy, safe, inexpensive, and simple means to improve sleep quality.

In the same line, Passos et al[22] divided patients with primary chronic insomnia into four groups (three experimental and one control). The experimental groups performed three different exercise programs (moderate aerobic exercises for 40 min, heavy aerobic exercises for 60 min, or moderate strengthening exercises), four days per week for four weeks. Only moderate exercise program resulted in the reduction of sleep onset latency and wake time after sleep onset by 45% and 36%, respectively, while increasing total sleep time by 21% and improving sleep efficiency by 18%. Additionally, Montgomery and Dennis[23] examined the effectiveness of moderate aerobic exercise and found significant improvement in the total sleep duration and sleep onset latency and scores on a scale of global sleep quality.

In contrast, Youngstedt and colleagues[24] found no significant difference in sleep onset latency or wakefulness after sleep onset after prolonged vigorous exercise for three hours, 30 min. before bedtime. Along the same line, Godwillas[25] examined the effect of late day exercise on insomnia and found that exercise disturbed sleep. The difference in the results between the present study and those of Youngstedt and Godwillas is most likely due to the difference in the type and time of exercise, as in the current study, patients performed moderate aerobic exercises six hours before bedtime. Other research was carried out by Tworoger et al[26] who investigated the effect of a moderate intensity treadmill walking and stationary bicycling for 45 min twice a day (morning and evening) and found no significant difference between morning and evening exercise on sleep quality.

The positive association between aerobic exercise and insomnia improvement could be explained by blood sugar and metabolism regulation. Such regulation is involved in the sleep–wake cycle. When metabolism increases during exercise, it then continues to run high even at rest. The heart beats more rapidly in order to deliver blood and oxygen to muscles so they can go through recovery process. Such process, along with the significant body exhaustion, can potentially improve insomnia[27]. The timing of exercise (late versus early evening) can also affect one's ability to fall asleep. It was proposed that exercise-induced sleep is mediated by cytokines which increase the non rapid eye movement sleep phase[28]. In addition, regular exercises improve sleep because insomniacs have increased levels of stress hormones in their blood, suggesting that they are abnormally sensitive to stress. The higher the stress hormone levels, the worse sleep is. Exercise initially increases such hormones but several hours after a workout, they decrease[29]. Such reports are in agreement with what was found in the current work where aerobic exercises were practiced afternoon or early evening several hours before bedtime thus providing enough time of stress hormones to decrease.Moreover, circadian sleep rhythm relates closely to body temperature and research implicates the involvement of the anterior hypothalamus in both heat loss and sleep mechanisms. Falling body temperature precedes the onset of sleep. In that respect, researchers have hypothesized that increasing body temperature before bedtime can activate both heat loss and associated sleep mechanisms. Notably, exercise raises body temperature more effectively than any other stimulus[30].Significant difference was also shown in the test parameters at the 2nd week compared to those at the 4th week of exercises in favor to the 4th week. This indicates that, to obtain positive effect, exercises should be performed for at least four weeks. Finally, as the study sample was small, further large scale studies are needed using various exercise maneuvers and timing in order to acknowledge aerobic exercise for insomniacs.

Conclusion

Moderate aerobic exercise was effective in improving symptoms of chronic primary insomnia and may be prescribed as an alternative treatment to drugs in the future.

References

1. *Shochat T, Umphress J, Israel AG, Ancoli-Israel S. Insomnia in primary care patients. Sleep 1999; 22 Suppl 2: S359-65.*

2. *Van Houdenhove L, Buyse B, Gabriëls L, Van den Bergh O. Treating primary insomnia: clinical effectiveness and predictors of outcomes on sleep, daytime function and health-related quality of life. J Clin Psychol Med Settings 2001; 18(3): 312-21.*

3. *National Heart, Lung and Blood Institute Working Group on Insomnia. Insomnia: assessment and management in primary care. Am Fam Physician 1999; 59: 3029–38.*

4. *Ohayon MM. Epidemiology of insomnia: What we know and what we still need to learn. Sleep Med Rev 1999; 6: 97-111.*

5. *Schenck H. Sleep: The mysteries, the problems, and the solutions, 1st ed. Penguin Group, New York, 2007, pp. 3-14.*

6. *Silber MH. Clinical practice: Chronic insomnia. N Engl J Med 2005; 353(8): 803–10.*

7. *Katz DA, McHorney CA. The relationship between insomnia and health-related quality of life in patients with chronic illness. J Fam Pract 2002; 51: 229–35.*

8. *Breslau N, Roth T, Rosenthal L, Andreski P. Sleep disturbances and psychiatric disorders: A longitudinal epidemiological study of young adults. Biol Psychiatry 1996 ; 39: 411–8.*

9. *Morin C, Espie C. Insomnia: A clinical guide to assessment and treatment, 1st edn. Kluwer academic/ Plenum, New York, 2003, pp. 16.*

10. *Johns MW. A new method for measuring daytime sleepiness: The Epworth sleepiness scale. Sleep 1991; 14: 540-5.*

11. *Perlis M, Jungquist C, Smith M, Posner D. Cognitive behavioral treatment of insomnia: A session-by-session guide, 1st ed. Springer, New York, 2005, pp. 1.*

12. *Mindell JA & Owens JA. A Clinical guide to pediatric sleep: Diagnosis &management of sleep problems, 2nd ed. Lippincatt Williams & wilkins, Philadelphia, 2010, pp. 293-4.*

13. *Idzikowski C. Beating insomnia: How to get a good night s sleep, 1st ed. Gill & Macmillan Ltd. Ireland, 2010, pp. 120-1.*

14. *Khanna A, Paul M, Sandhu J. Efficacy of two relaxation techniques in reducing pulse rate among highly stressed females. Calicut Medical Journal 2010; 5 (5): 2-7.*

15. *Gormley J, Hussey J. Exercise therapy prevention and treatment of disease, 1st edn. Blackwell, Oxford, 2005, pp. 82.*

16. *Sherrill DL, Kotchou K, Quan SF. Association of physical activity and human sleep disorders. Arch Intern Med 1998; 158: 1894-8.*

17. *Tanaka H, Taira k, Arakawa M, Uraski C, Yamamoto Y, Okuma H, Uezu E, Sugita Y and Shirakawa S. Short naps and exercise improve sleep quality and mental health in the elderly. Psychiatry Clin Neurosci 2002; 56: 233- 4.*

18. *Youngstedt SD. Effects of exercise on sleep. Clin Sports Med 2005; 24: 355-65.*

19. *Bain KT. Management of chronic insomnia in elderly persons. Am J Geriatr Pharmacother 2006; 4: 168–92.*

20. *Kapella MC, Herdegen JJ, Perlis ML, Shaver JL, Larson JL, Law JA, Carley DW. Cognitive behavioral therapy for insomnia comorbid with COPD is feasible with preliminary evidence of positive sleep and fatigue effects. Int J Chron Obstruct Pulmon Dis 2011; 6: 625-35.*

21. *Sorscher AJ. Sleep and the Family Doctor: Time to Lead. J Am Board Fam Med 2011; 24: 133-5.*

22. *Passos GS, Poyares D, Santana MG, Garbuio SA, Tufik S, Mello MT. Moderate exercise can improve the sleep quality of insomnia patients. J Clin Sleep Med 2008; 6: 270-5.*

23. *Montgomery P, Dennis A. Physical exercise for sleep problems in adults aged 60+. The Cochrane Collaboration 2009; 44: 1465-9.*

24. *Youngstedt SD, Kripke DF, Elliott JA. Is sleep disturbed by vigorous late-night exercise? Med Sci Sports Exerc 1999; 31: 864-9.*

25. *Godwillas A. Can late-day exercises cause insomnia? J Clin Sleep Med 2007; 54: 744-50.*

26. *Tworoger SS, Yasui Y, Vitiello MV, Schwartz RS, Urich CM, Aiello EJ, Irwin ML, Bowen D, Potter JD and McTiernan A. Effects of a yearlong moderate-intensity exercise and a stretching intervention on sleep quality in postmenopausal women. Sleep 2003; 26: 830-6.*

27. *Paul M. Aerobic exercise relieves insomnia. Institute for women's health research 2010; 15: 321-3.*

28. *Cappuccio FP, Miller MA, Lockley SW. Sleep, health & society: from aetiolgy to public health, 1st ed. Oxford University press, New York, 2010, pp. 245.*

29. *Morgenthaler T. Late-day Exercise Can It Cause Insomnia? Mayo Foundation for Medical Education and Research 2009; 64(5): 321-5.*

30. *Spirduso W, Poon L, Chodzko W, Chodzko Z. Exercise and its mediating effects on cognition, 2nd ed. Human Kinetics, Europe, 2007, pp. 137-8.*

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Appendix

Sleep Diary

Please shade the box to show when you slept each day and night. For example, if you slept from 2 pm until 4 pm and then again from midnight until 4am, the record would look like this:

Total hours slept: 6 hrs.

Please list all of you medications below (include herbal and over the counter (OTC) medications that you take):

Please add any notes that you feel are important to know about your sleep: / / until / /

Monday

Daytime Night-time

Hours slept:

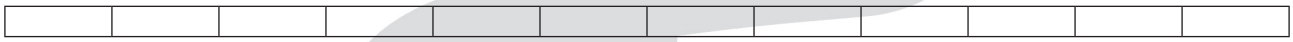
Tuesday

Daytime Night-time

Hours slept:

Wednesday

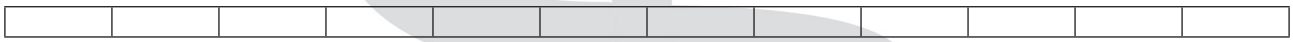
Daytime Night-time



Hours slept:

Thursday

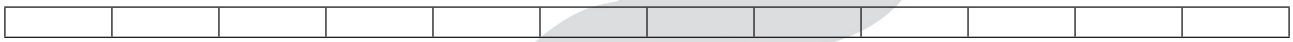
Daytime Night-time



Hours slept:

Friday

Daytime Night-time



Hours slept:

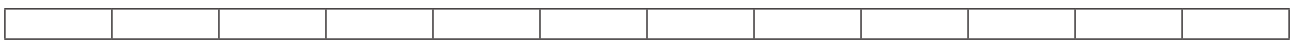
Saturday

Daytime Night-time

Hours slept:

Sunday

Daytime Night-time



Hours slept:

Clinical severity of sickle cell anemia alone and sickle cell diseases with thalassemias

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Abstract

Background: We tried to understand whether or not there is a relief of clinical severity in sickle cell diseases (SCDs) with thalassemias against sickle cell anemia (SCA) alone.

Methods: SCA cases alone and SCDs cases with thalassemias were compaired in between according to chronic obstructive pulmonary disease, pulmonary hypertension, digital clubbing, leg ulcers, chronic kidney disease, cirrhosis, stroke, autosplenectomy, and mortality.

Results: The study included 218 cases with SCA alone and 58 cases with SCDs with alpha- or beta-thalassemias. White blood cell (wbc) and platelet counts were significantly higher in SCA cases alone, 16.285/μL versus 13.215/μL (p= 0.000) and 469.500/μL versus 389.840/μL (p= 0.007), respectively. Whereas, the mean hematoctit value was significantly lower in the SCA cases alone (22.5% versus 26.0%, p= 0.000). Although, prevalences of most of the studied pathologies and mortality were higher and the mean age of mortality was lower in SCA cases alone, the differences were only significant for pulmonary hypertension (13.3% versus 3.4%, p<0.05), digital clubbing (7.3% versus 0.0%, p<0.05), and autosplenectomy (52.7% versus 22.4%, p<0.001), probably due to the small sample size of the SCDs with thalassemias cases.

Conclusion: The relatively suppressed hemoglobin S synthesis in SCDs with thalassemias cases may decrease sickle cell-induced chronic endothelial damage and inflammation, and all the terminal consequences of SCDs. The higher wbc and platelet counts and the lower mean hematocrit value may also indicate the severity of chronic inflammatory process and secondary anemia in the SCA cases alone.

Key words: Sickle cell anemia, sickle cell diseases, thalassemias, clinical severity.

Introduction

Sickle cell diseases (SCDs) are characterized by sickle-shaped erythrocytes which is caused by homozygous inheritance of the hemoglobin S (Hb S). They are chronic hemolytic anemias including sickle cell anemia (SCA) alone and sickle cell-hemoglobin C, sickle cell-beta-thalassemia, and sickle cell-alpha-thalassemia diseases. As in thalassemias, they are especially common in malaria-stricken areas of the world. The responsible allele is autosomal recessive located on the short arm of the chromosome 11. Glutamic acid is replaced with a less polar amino acid, valine, in the sixth position of the beta chain of the Hb S. Under stressful conditions including cold, exercise, pregnancy, infections, emotional distress, and hypoxia, presence of a less polar amino acid promotes polymerisation of the Hb S, which distorts erythrocyte into a sickle shape and decreases its elasticity. The decreased elasticity of the erythrocytes is the central pathology of the disease, since the normal erythrocytes can deform to pass through capillaries easily. Hb S causes erythrocytes to change their normal elastic and biconcave disc shaped structures to a hard and sickle shaped bodies. The sickling process is probably present in whole life period of the human being, but it is exaggerated during the above stressful conditions of the body. The erythrocytes can take their normal elastic shapes after normalization of the stressful conditions, but after repeated cycles of sickling and unsickling, they become a hard body, permanently, and the chronic endothelial damage and hemolysis develop. So lifespan of the erythrocytes decreases to 15-25 days. Chronic endothelial damage induced tissue ischemia and infactions, even in the absence of a prominent vascular occlusion, are the final consequences of the disease, so the life expectancy the homozygotes is decreased by 25 to 30 years (1). We tried to un-

derstand whether or not there is a relief of clinical severity in SCDs with thalassemias against SCA alone in the present study.

Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and November 2012. All patients with SCDs were enrolled into the study. SCDs are diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography (HPLC). Thalassemias are diagnosed by the mean corpuscular volume, serum iron, total iron-binding capacity, serum ferritin, hemoglobin electrophoresis via HPLC, and genetic testing in required cases. Medical histories including smoking habit, regular alcohol consumption, leg ulcers, stroke, and operations were learnt. Cases with a history of one pack-year were accepted as smokers, and alcohol consumers at least once a day were accepted as regular alcohol consumers. Additionally, serum creatinine value on three occasions, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, 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 crises were treated at first, and then spirometric pulmonary function tests to diagnose chronic obstructive pulmonary disease (COPD), the Doppler echocardiography to measure the systolic blood pressure (BP) of pulmonary artery, and renal and hepatic function tests were performed on a silent phase. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% (2). Systolic BP of the pulmonary artery at and above 40mmHg during the silent phase is accepted as pulmonary hypertension (3). Chronic kidney disease (CKD) was diagnosed with a permanently elevated serum creatinine level which is higher than 1.2 mg/dL on the silent phase. Cases with renal transplantation were also put into the CKD 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 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 (4,5). Eventually, SCA cases alone and SCDs with thalassemias were compaired according to gender distribution, mean age, white blood cell (wbc) count, platelet count, hematocrit value, smoking habit, regular alcohol consumption, COPD, pulmonary hypertension, digital clubbing, leg ulcers, CKD, cirrhosis, stroke, autosplenectomy, and mortality. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 218 cases with SCA (108 females) alone and 58 cases with SCDs with thalassemias (25 females) (Table 1). There were 46 cases with beta- and 12 cases with alpha-thalassemias. The wbc and platelet counts were significantly higher in SCA cases alone, 16.285/μL versus 13.215/μL (p= 0.000) and 469.500/μL versus 389.840/μL (p= 0.007), respectively. Whereas, the mean hematoctit value was significantly lower in the SCA cases alone (22.5% versus 26.0%, p= 0.000). There was not any patient with regular alcohol consumption among the study cases. Although antiHCV was positive in two of the cirrhotic cases, HCV RNA was negative via polymerase chain reaction method in both. Although, most of the studied pathologies and mortality were higher in the SCA cases alone, the differences were only significant for pulmonary hypertension (13.3% versus 3.4%, p<0.05), digital clubbing (7.3% versus 0.0%, p<0.05), and autosplenectomy (52.7% versus 22.4%, p<0.001), probably due to the small sample size of the SCDs with thalassemias group. The mean ages of mortality were also lower in the SCA group, nonsignificantly (27.6 versus 33.0 years, p>0.05), probably due to the same reason again (Table 2).

Discussion

Thalassemias are chronic hemolytic anemias, too and 1.6% of the population are heterozygous for alpha- or beta-thalassemias in the world (6). They are autosomal recessively inherited disorders as in

Table 1. Characteristic features of the study cases

Variables	SCA* cases	SCDs† with thalassemias cases	p-value
Number	218	58	
Female ratio	49.5% (108)	43.1% (25)	ns‡
Mean age (year)	29.2 ± 9.3 (13-59)	28.7 ± 10.2 (17-54)	ns
White blood cell count (µL)	16.285 ± 6.127 (6.500-39.200)	13.215 ± 7.401 (2.900-33.100)	0.000
Hematocrit value (%)	22.5 ± 4.4 (8-39)	26.0 ± 5.5 (11-35)	0.000
Platelet count (µL)	469.500±198.808 (56.000-1.561.000)	389.840±253.726 (48.800-919.000)	0.007

**Sickle cell anemia †Sickle cell diseases ‡Nonsignificant (p>0.05)*

Table 2. Associated pathologies of the study cases

Variables	SCA* cases	SCDs† with thalassemias cases	p-value
Smoking	10.0% (22)	8.6% (5)	ns‡
COPD§	6.8% (15)	6.8% (4)	ns
Pulmonary hypertension	13.3% (29)	3.4% (2)	<0.05
Digital clubbing	7.3% (16)	0.0% (0)	<0.05
Leg ulcers	11.0% (24)	10.3% (6)	ns
CKD¶	9.1% (20)	5.1% (3)	ns
Cirrhosis	6.8% (15)	3.4% (2)	ns
Stroke	5.9% (13)	3.4% (2)	ns
Autosplenectomy	52.7% (115)	22.4% (13)	<0.001
Mortality	5.5% (12)	5.1% (3)	ns
Mean age of mortality (year)	27.6 ± 8.4 (19-45)	33.0 ± 12.1 (19-41)	ns

**Sickle cell anemia †Sickle cell diseases ‡Nonsignificant (p>0.05) §Chronic obstructive pulmonary disease ¶Chronic kidney disease*

SCA. It results from unbalanced Hb synthesis caused by decreased production of at least one globin polypeptide chain (alpha, beta or delta) that build up the normal Hb. HbA1 is composed of two pairs of alpha and beta chains that represents about 97% of total Hb in adults. Alpha-thalassemias result from decreased alpha chain synthesis and beta-thalassemias from decreased beta chain synthesis. Because of the mild to moderate anemia induced tissue hypoxia, bone marrow hyperactivity secondary to the chronic hemolytic process, splenic hyperactivity particularly in huge splenomegaly cases, and cardiopulmonary hyperactivity, growth retardation is possible in thalassemia cases. Beta globin chains are encoded by a single gene on chromosome 11, whereas alpha chains by two closely linked genes on chromosome 16. Thus in a normal person with two copies of each chromosome, there are two alleles encoding the beta, whereas four alleles encoding the alpha chains. The fact can explain the predominance of beta-thalassemia over alpha-thalassemia cases in the present study. Heterozygotes for a single gene defect are usually free of clinical abnormalities. Heterozygotes for a double gene defect or homozygotes for a single gene defect tend to manifest a clinical picture similar to heterozygotes for the beta-thalassemia. Inheritance of a single gene defect plus a double gene defect more severely impairs alpha chain synthesis, and alpha chain deficiency results in the formation of tetramers of excess beta chains (Hb H) in adults or gamma chains (Bart's Hb) in infants. Homozygosity for the double gene defect is lethal since Hb that lacks alpha globin chains can not transport oxygen. So the thalassemia major and intermedia cases are actually beta-thalassemia cases in the clinic.

Chronic endothelial damage and associated atherosclerosis may be the major health problem of the human being, since they may be the main cause of aging and organ failures. Probably atherosclerosis is an irreversible process that accelerated by many factors. Smoking, dyslipidemia, obesity, diabetes mellitus (DM), hypertension (HT), and various inflammatory and infectious disorders may be the accelerating causes of the systemic process. Such preventable causes of the systemic atherosclerosis are mainly collected under the heading of metabolic syndrome (7-11), which is characterized by

reversible risk factors including overweight, dyslipidemia, elevated BP, and insulin resistance for the development of terminal diseases such as obesity, HT, DM, peripheric artery disease, coronary heart disease, COPD, cirrhosis, CKD, and stroke (12,13). Due to the hard and sickle shaped erythrocytes, SCDs may also be a strong risk factor for chronic endothelial damage and atherosclerosis that may cause early aging and death.

Painful crises are the most common and disabling symptoms of the SCDs. Although some authors reported that pain itself may not be directly life threatening (14), infections are the most common precipitating factors of the painful crises. Additionally, the crises probably develop due to the tissue hypoxia which is secondary to an accelerated endothelial damage induced by an accelerated sickling process. So the risk of mortality is significantly higher during the crises. On the other hand, whether leukocytosis contributes to the pathogenesis of the painful crises by releasing cytotoxic enzymes is unknown. The adverse actions of neutrophils on endothelium are of particular interest with regard to the cerebrovascular diseases in SCDs. For example, leukocytosis even in the absence of any infection was an independent predictor of the severity of the disease (15), and it was associated with the risk of stroke in a cohort of Jamaican patients (16). Occlusions of vasculature of the bone marrow, bone infarctions, releasing of inflammatory mediators, and activation of afferent nerves may take role in the pathophysiology of the intolerable pain. Due to the severity of pain, narcotic analgesics are usually required to control them (17), but according to our practice, erythrocyte transfusions may be highly significant in severe painful crises both to relieve severe pain and to prevent sudden death that may develop secondary to acute chest syndrome and sepsis induced multiorgan failures on chronic background of SCDs.

Because of the repeated infarctions and subsequent fibrosis, the spleen is commonly very small in adults. Eventually, a functional and anatomic asplenism develop due to the decreased antibody production, prevented opsonization, and reticuloendothelial dysfunction. Similarly, parallel to the higher wbc and platelet counts and the lower mean hematocrit value, the significantly higher prevalence of autosplenectomy may be another indicator of the severity of chronic inflammatory process in SCA cases alone. The significantly lower wbc and platelet counts in SCDs with thalassemias group could not be explained by hypersplenism which is much more common in such cases, since the mean hematocrit value was significantly higher as an opposite finding to the hypersplenism in them. The 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 (15). In another study, the peak incidence of death among children occured between 1 and 3 years of age, and the deaths among patients less than 20 years were predominantly caused by pneumococcal sepsis (18). As also observed in the present study, SCDs cases, even those who appear relatively fit, are susceptible to sepsis induced multiorgan failure and sudden death during acute crises on the ground of generalized immunosuppression.

SCDs can affect nearly all organ systems of the body (19-20). Even there was a patient with sickle cell retinopathy induced severe vision loss among our study cases. Eventually, the mean survival was 42 years for males and 48 years for females in the literature (8), whereas it was 27 and 33 years in the two groups in the present study. The great differences about the survival between the literature and Turkey should be searched with further studies, but may be secondary to the initiation of hydroxyurea treatment in early life in developed countries. As a result of such a great variety of clinical presentation, it is not surprising to see that the mean body weight and body mass index (BMI) were significantly retarded in the SCDs cases (21). On the other hand, as an opposite finding to some other reports (22-23), the mean heights were nearly similar in the SCDs and control cases in the above study (21). Probably due to the lower mean body 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 (21), which can be explained by the metabolic syndrome (24-26).

As a conclusion, the relatively suppressed hemoglobin S synthesis in SCDs with thalassemias cases may decrease sickle cell-induced chronic endothelial damage and inflammation, and all the terminal consequences of SCDs. The higher wbc and platelet counts and the lower mean hematocrit value may also indicate the severity of chronic inflammatory process and secondary anemia in SCA cases alone.

References

1. *Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 1994; 330: 1639-1644.*

2. *Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease 2010. Global initiative for chronic obstructive lung disease (GOLD).*

3. *Fisher MR, Forfia PR, Chamera E, Housten-Harris T, Champion HC, Girgis RE, et al. Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. Am J Respir Crit Care Med 2009; 179: 615-621.*

4. *Schamroth L. Personal experience. S Afr Med J 1976; 50: 297-300.*

5. *Vandemergel X, Renneboog B. Prevalence, aetiologies and significance of clubbing in a department of general internal medicine. Eur J Intern Med 2008; 19: 325-329.*

6. *Angastiniotis M, Modell B. Global epidemiology of hemoglobin disorders. Ann N Y Acad Sci 1998; 850: 251-269.*

7. *Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005; 365: 1415-1428.*

8. *Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109: 433-438.*

9. *Helvaci MR, Kaya H, Gundogdu M. Association of increased triglyceride levels in metabolic syndrome with coronary artery disease. Pak J Med Sci 2010; 26: 667-672.*

10. *Helvaci MR, Kaya H, Borazan A, Ozer C, Seyhanli M, Yalcin A. Metformin and parameters of physical health. Intern Med 2008; 47: 697-703.*

11. *Helvaci MR, Seyhanli M. What a high prevalence of white coat hypertension in society! Intern Med 2006; 45: 671-674.*

12. *Helvaci MR, Aydin LY, Aydin Y. Chronic obstructive pulmonary disease may be one of the terminal end points of metabolic syndrome. Pak J Med Sci 2012; 28: 376-379.*

13. *Anderson RN, Smith BL. Deaths: leading causes for 2001. Natl Vital Stat Rep 2003; 52: 1-85.*

14. *Parfrey NA, Moore W, Hutchins GM. Is pain crisis a cause of death in sickle cell disease? Am J Clin Pathol 1985; 84: 209-212.*

15. *Miller ST, Sleeper LA, Pegelow CH, Enos LE, Wang WC, Weiner SJ, et al. Prediction of adverse outcomes in children with sickle cell disease. N Engl J Med 2000; 342: 83-89.*

16. *Balkaran B, Char G, Morris JS, Thomas PW, Serjeant BE, Serjeant GR. Stroke in a cohort of patients with homozygous sickle cell disease. J Pediatr 1992; 120: 360-366.*

17. *Cole TB, Sprinkle RH, Smith SJ, Buchanan GR. Intravenous narcotic therapy for children with severe sickle cell pain crisis. Am J Dis Child 1986; 140: 1255-1259.*

18. *Leikin SL, Gallagher D, Kinney TR, Sloane D, Klug P, Rida W. Mortality in children and adolescents with sickle cell disease. Cooperative Study of Sickle Cell Disease. Pediatrics 1989; 84: 500-508.*

19. *Haupt HM, Moore GW, Bauer TW, Hutchins GM. The lung in sickle cell disease. Chest 1982; 81: 332-337.*

20. *Shapiro MP, Hayes JA. Fat embolism in sickle cell disease. Report of a case with brief review of the literature. Arch Intern Med 1984; 144: 181-182.*

21. *Helvaci MR, Kaya H. Effect of sickle cell diseases on height and weight. Pak J Med Sci 2011; 27: 361-364.*

22. *Al-Saqladi AW, Cipolotti R, Fijnvandraat K, Brabin BJ. Growth and nutritional status of children with homozygous sickle cell disease. Ann Trop Paediatr 2008; 28: 165-189.*

23. *Zemel BS, Kawchak DA, Ohene-Frempong K, Schall JI, Stallings VA. Effects of delayed pubertal development, nutritional status, and disease severity on longitudinal patterns of growth failure in children with sickle cell disease. Pediatr Res 2007; 61: 607-613.*

24. *Helvaci MR, Kaya H, Yalcin A, Kuvandik G. Prevalence of white coat hypertension in underweight and overweight subjects. Int Heart J 2007; 48: 605-613.*

25. *Helvaci MR, Kaya H, Duru M, Yalcin A. What is the relationship between white coat hypertension and dyslipidemia ? Int Heart J 2008; 49: 87-93.*

26. *Helvaci MR, Kaya H, Sevinc A, Camci C. Body weight and white coat hypertension. Pak J Med Sci 2009; 25(6): 916-921.*

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Selected markers of bone metabolism in periodontal diseases

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Abstract

The bone of an adult undergoes a constant remodelling. The bone formation and resorption processes compensate each other. In pathological conditions the resorption can overbalance the bone formation. Many enzymes and cells participate in this process. Periodontal diseases can lead to bone resorption, slow loss of the bone structure and as a result the tooth loss. The clinical medicine has been dealing with the problem of bone metabolism for many years. On the other hand, few research studies on biochemical markers evaluating the level of bone resorption and formation can be found in the dental literature. Therefore we decided to evaluate the level of two bone formation markers (osteocalcin and PINP) and one resorption marker (CTx) in patients with the periodontal diseases. 29 patients with the periodontal disease (chronic periodontitis) and 28 healthy volunteers (without any periodontal disease) were examined. The clinical periodontal status was assessed. The abovementioned bone metabolism markers were investigated using the electrochemiluminescence method. A statistical analysis did not show any differences in the levels of these markers. However, a positive correlation between the osteocalcin, the PINP and the CTx was found.

Key words: Periodontal disease, bone resorption and formation markers.

Introduction

The bone in adult individuals undergoes a constant remodelling. In normal conditions there is a dynamic equilibrium between the bone resorption and formation. The bone turnover markers are enzymes and proteins released to the circulation during bone formation and fragments of the bone matrix developed during bone resorption (1,2). Using biochemical methods the bone metabolism indices can be evaluated. They are divided into two groups: bone formation and resorption markers (3).

The bone formation markers include the osteocalcin (BGP) (4,5). It is a specific, non-collagenous bone matrix protein which is synthesized by osteoblasts and odontoblasts. A portion of the BGP which has not been deposited in the bone matrix is released to the blood where it can be detected. The function of the osteocalcin in the bone tissue has not been completely explained. The in vivo and in vitro studies conducted so far suggested its participation in the chemotaxis of osteoclasts, their migration and adhesion to the mineralized bone surface. This protein influences the nature of the morphotic structure of osteoclasts and their resorptive function (6,7,8). The calcium ion affinity of the osteocalcin enables it to influence the process of bone matrix mineralization. About 10% of synthesized osteocalcin is released to the circulation, and then excreted with the urine. The concentration of osteocalcin can be determined in the serum and the urine using the radioimmunological and immunoenzymatic method (5,9). This is a sensitive specific test which can evaluate the bone formation. The second marker evaluating the bone formation is the Procollagen type I N-terminal Propeptide (PINP). It is released from the Procollagen type I Aminoterminal Propeptide. It can be determined using the radioimmunological method (10, 11, 12).

From many tests used for the evaluation of resorption the C-terminal cross-linking telopeptide of the type I collagen alpha chain (CTx) is worth mentioning (13). The CTx assay in the serum allows obtaining a positive response to therapy as early as within 4-8 weeks from its beginning. In case of the osteopenia and osteoporosis therapy it provides the

information whether or not the chosen method of treatment is right or how it can be corrected. The CTx is released during the degradation of collagen and excreted through the kidneys. This marker is not specific for the bone tissue, yet it is more diagnostically sensitive and specific than other indices, such as pyridinoline and deoxypyridinoline (12).

Aim of the paper

The authors decided to evaluate how bone turnover metabolites act in the periodontal diseases and to compare these values with the level of values obtained in generally healthy individuals without any periodontal disease.

The biochemical tests concerned two bone formation markers: the osteocalcin and the PINP as well as the bone resorption marker (CTx).

Material and methods

The clinical population consisted of periodontal disease patients treated in a periodontal clinic (29 individuals) aged between 22 and 74 years, 51.4 on average. The approval of the Bioethical Committee to conduct the study was obtained. The patients agreed in writing to the clinical examination and to taking a blood sample for the biochemical tests. The reference population consisted of 28 generally healthy individuals without any periodontal disease, the staff of dentistry departments aged between 28 and 63 years, average age 41.5 years.

The periodontal examination comprised:

1. The measurement of the simplified O'Leary Plaque Index (PI) (the sum of all surfaces with dental plaque/ sum of all examined surfaces x 100%).
2. The assessment of the Ainamo and Bay Bleeding Index (BI): the sum of all surfaces in which bleeding occurred/the sum of all examined surfaces x100%), the bleeding index was measured at six measuring points – mesially, medially and distally for the labial/ buccal and lingual/palatal surface of all teeth.
3. The clinical gingival status was evaluated using the Löe and Silness Gingival Index (GI) by assessing the gingiva with all teeth present in the oral cavity, by examining four gingival surfaces surrounding a tooth, i.e. the buccal, lingual, mesial and distal surfaces. The gingival status evaluation criteria in this index are entirely based on clinical qualitative changes in the gingival tissues and are as follows: 0- the absence of gingivitis symptoms, the absence of any pathological change in the colour of the gums, 1- a mild gingivitis with a small change in the colour of the gums, a mild change in the structure of the gingival tissue, the absence of bleeding on probing, 2- a moderate gingivitis: redness, swelling, shiny appearance and hypertrophy of the gums, bleeding on pressure or probing, 3- a serious gingivitis manifesting itself in a considerable redness, swelling, ulceration and the susceptibility to spontaneous bleeding.
4. The average Pocket Depth (PD), a value measured from the bottom of a periodontal pocket to the edge of the free gingiva, given in mm.
5. The average level of the Clinical Attachment Loss (CAL), a value measured from the bottom of a periodontal pocket to the cement-enamel junction, given in mm.

The PD and CAL values were measured at six measuring points.

For the evaluation of the periodontal status (items 4 and 5) the PD and CAL percentages with the value equal to or higher than 6 mm were used.

The biochemical tests were carried out at the Department of Laboratory Paediatric Diagnostics of the Medical University of Bialystok. The laboratory tests concerned two bone formation markers: the osteocalcin and the PINP (Procollagen type I N-terminal Propeptide) as well as the bone resorption marker - CTx (C-terminal cross-linking telopeptide of the type I collagen alpha chain, B-Cross Laps). The osteocalcin, PINP and B-Cross Laps (CTx) were determined by means of the electrochemiluminescence method using streptavidin- and ruthenium compound-coated magnetic particles on the Cobas e411 apparatus of Hitachi.

Statistical tests

For the evaluation of the level of bone metabolism markers in the blood serum the IBM SPSS Statistics 20.0 program was used. The analysed parameters were described by specifying the arithme-

tic mean (X), the standard deviation (SD), the minimum and maximum values, and the percentages (%). The Mann-Whitney Test and the Spearman's nonparametric correlations were used. The results of the statistical tests were found significant at the level of $p<0.05$.

Results

Clinical periodontal examination

Chronic periodontitis was diagnosed in all patients qualified for the study. The periodontal status is shown in Table 1. The Plaque Index was between 23.7% and 99.3% (mean 60.24%). The BI was between 9.8 and 94.79% (mean 66.27%). The Gingival Index was between 0.49 and 3.0 (mean 2.15) – this index evaluates the gingiva only. In the evaluation of the PD and the CAL the percentage of teeth where the attachment loss and the depth of periodontal pockets was equal to or more than 6 mm was taken into consideration. Such progression of the periodontal destruction affected all subjects. Advanced periodontal diseases were found in the range from 3.84% to 100% (mean 47.78%).

Laboratory test results

The level of concentration of the osteocalcin in the control group was 22.31 ng/ml, and in the periodontal disease group 23.5 ng/ml. The differences were not statistically significant. The PINP concentration in the control group was 46.71 ng/ml, whereas in the periodontal disease group this value was slightly lower – 43.63 ng/ml. The differences were not statistically significant. The level of B-Cross Laps in the periodontal disease group of 0.23 ng/ml was slightly higher than in the control group – 0.19 ng/ml (Table 2). The differences were not statistically significant. However, a positive correlation between the osteocalcin, the PINP and the B-Cross Laps occurred both in the control group (Table 3) and in the periodontal disease group (Table 4). A positive correlation occurred also in both groups together (Table 5). The correlation did not depend on the degree of progression of the periodontal disease or the number of teeth present in the oral cavity (Table 6).

Table 1. Periodontal status in the clinical population

Group	n	Mean	Stand. deviat.	Minimum	Median	Maximum
PI [%]	29	60.24	21.4	23.7	59	99.3
GI	29	2.15	0.69	0.49	2.25	3.00
BI[%]	29	66.27	21.82	9.80	71.25	94.79
PD, CAL [%]	29	47.78	27.57	3.84	41.66	100.00
Number of teeth	29	22.72	4.95	12.00	23.00	28.00

Table 2. Comparison of the level of osteocalcin, PINP and CTx in the clinical population (with the periodontal disease) and the control group (ng/ml)

Group		n	Mean	Stand. dev.	Minimum	Median	Maximum	p*
Osteocalcin	Control	28	22.31	7.95	11.54	20.79	45.55	0.779
	Perio. dis.	29	23.54	10.39	12.67	21.15	67.33	
	Total	57	22.94	9.23	11.54	20.96	67.33	
PINP	Control	28	46.71	19.98	21.19	40.57	106.10	0.744
	Perio. dis.	29	43.63	14.75	21.50	40.71	82.66	
	Total	57	45.12	17.38	21.19	40.57	106.10	
CROSSL	Control	28	0.19	0.14	0.05	0.16	0.78	0.455
	Perio. dis.	29	0.23	0.21	0.01	0.17	1.19	
	Total	58	0.21	0.18	0.01	0.17	1.19	

** Mann-Whitney Test*

Table 3. Spearman's nonparametric correlations in the control group among osteocalcin, PINP and CROSSL

Group		Osteocalcin	PINP	CROSSL
Osteocalcin	r	1.00	0.84	0.78
	P		0.000	0.000
PINP	r	0.84	1.00	0.81
	p	0.000		0.000
CROSSL	r	0.78	0.81	1.00
	p	0.000	0.000	

Table 4. Spearman's nonparametric correlations in the periodontal disease group among osteocalcin, PINP and CROSSL

Group		Osteocalcin	PINP	CROSSL
Osteocalcin	r	1.00	0.65	0.55
	p		0.000	0.002
PINP	r	0.65	1.00	0.44
	p	0.000		0.014
CROSSL	r	0.55	0.44	1.00
	p	0.002	0.014	

Table 5. Spearman's nonparametric correlations in both groups together (control group and periodontal disease group)

Group		Osteocalcin	PINP	CROSSL
Osteocalcin	r	1.00	0.74	0.66
	p		0.000	0.000
PINP	r	0.74	1.00	0.61
	p	0.000		0.000
CROSSL	r	0.66	0.61	1.00
	p	0.000	0.000	

Table 6. Spearman's nonparametric correlations between the bone metabolism parameters and the periodontal status

Group		Osteocalcin	PINP	CROSSL
PI	r	0.07	0.03	0.00
	p	0.737	0.879	0.986
GI	r	-0.02	0.02	0.31
	p	0.920	0.934	0.097
BI	r	-0.08	0.00	0.18
	p	0.676	0.996	0.338
PD/CAL	r	-0.24	-0.26	-0.15
	p	0.201	0.171	0.427
Number of teeth	r	-0.22	0.17	-0.18
	p	0.258	0.387	0.344

Discussion

Undoubtedly, in the course of the periodontal disease there are periods of connective tissue collagen destruction, bone resorption and periods when these destruction processes are overbalanced by the periods of inhibition of the disease process, and even the periods of bone tissue reconstruction. The periodontal disease patients were qualified for the study when they reported for the specialist treatment. If a repeat test of the bone metabolism markers was conducted, it could pro-

vide an evaluation of the biochemical processes occurring in the alveolar bone and a prognosis of the treatment result. The clinical usability of bone markers has been documented and is accepted by doctors of different specialities. In medicine, the bone metabolism is evaluated in the monitoring of metabolic disorders (14).

The tests of the level of bone turnover markers are used in the diagnostics, treatment and monitoring of osteoporosis and osteopenia (3, 4, 9, 11). In orthopaedics, such tests are employed for the evaluation of bone healing after the use of endoprostheses (12). In the evaluation of cancer metastases, the bone turnover markers allow an early diagnosis of bone metastases of a breast, lung, thyroid, kidney and prostate cancer (10, 15,16). The publications evaluating the concentration of bone metabolism markers in the course of oral diseases: in the course of the mandibular fracture treatment (17,18,19) and in the orthodontic treatment, where as a result of the treatment with an active orthodontic appliance a remodelling of the alveolar process occurred (12,13), have appeared for some time. The Maxillofacial Surgery Centre of the Medical University of Bialystok used the test of the bone metabolism markers in the evaluation of the healing process of mandibular fractures in men depending on the treatment applied (17, 18, 19).

Our research is difficult to compare with the research of other authors concerning the level of these biochemical metabolites in the periodontal diseases because no such research has been conducted as yet. This method seems to complement the diagnostic methods which allow evaluating both the activity of the disease process and the treatment results. From the literature appears that in view of a broad range of normal values it is necessary to determine reference values for these indices (14). It is related to the fact that the level of bone turnover markers depends on the age, gender, and hormonal and nutritional status (14).

References

1. *Manolagas SC, Jilka RL. Bone marrow, cytokines and bone remodeling. N. Engl. J Med., 1995; 332: 305-311*
2. *Rodan GA. Introduction to bone biology. Bone. 1992; 13: 3-6.*
3. *Gamero P, Delmas P. New developments in biochemical markers for osteoporosis. Calciff. Tissue Int., 1996; 59: 2-9.*
4. *Delmas PD. Clinical use of biochemical markers of bone remodeling in use of biochemical markers of bone remodeling in osteoporosis. Bone, 1992; 13: 17-21.*
5. *Masters PW, Jones RG, Purves DA, et al. Commercial assays for serum osteocalcin give clinically discordant results. Clin. Chem., 1994; 40: 358-363.*
6. *Chenu C, Colucci S, Grano M, et al. Osteocalcin induces chemotaxis, secretion of matrix proteins and calcium-mediated intracellular signaling in human osteoclast-like cells. J. Cell Biol., 1994; 127: 1149-1158.*
7. *Young MF, Kerr MJ, Ibaraki K, et al. Structure, expression,and regulation of the major noncollagenous matrix proteins of bone. Clin. Orthop., 1992; 281: 275-293.*
8. *Grabowska SZ. Wybrane markery biochemiczne procesu przebudowy tkanki kostnej. Czas. Stomt. 49, 6: 436-440.*
9. *Delmas PD. Biochemical markers of bone turnover I: Theoretical considerations and clinical use in osteoporosis. Am.J. Med., 1993; 95: 5-11*
10. *Litwiniuk M, Kopczyński Z. Przydatność markerów obrotu kostnego w rozpoznawaniu i monitorowaniu przebiegu leczenia przerzutów nowotworowych do kości. Współ. Onkol. 2002; 6: 384-390.*
11. *Marcinkowska E. Biochemiczne markery przebudowy kości i ich przydatność do diagnostyki osteoporozy. Wiad. Lek.1992; 45: 17-18.*
12. *Kopczyński P. Możliwości zastosowania wybranych markierów obrotu kostnego w stomatologii. Nowiny Lekarskie 2004; 73,4: 318-320.*
13. *Kopczyński P. Ocena zmian stężenia markierów obrotu kostnego u pacjentów ze zgryzem krzyżowym leczonych aparatem quadhelix. Dental Forum, 2010; 38, 1: 59-62.*
14. *Gajewska J, Ambroszkiewicz J, Laskowska-Klita T. Wybrane markery obrotu kostnego w surowicy krwi dzieci w zależności od wieku i płci. Wiadomości Lekarskie, 2005; 58, 9-10: 476-480.*

15. *Fontana A, Delmas PD. Markers of bone turnover in bone metastases. Cancer, 2000; 88: 2952-60.*

16. *Lorente JA, Morote J, Raventos C, et al. Clinical efficacy of bone alkaline phosphoatase and prostate specific antigen in the diagnostics of bone metastasis in prostate cancer. J. Urol., 1996; 155: 1348-51*

17. *Borys J, Grabowska SZ, Antonowicz B, et al. N-końcowy propeptyd prokolagenu typu III (PIII NP.) w ocenie gojenia złamań żuchwy u mężczyzn. Czas. Stomat. 2004; 57, 4: 246-254.*

18. *Borys J, Grabowska SZ, Antonowicz B, et al. Stężenie C-końcowego telopeptydu kolagenu typu I (ICTP) w surowicy mężczyzn w przebiegu gojenia złamań żuchwy. Czas. Stomatol. 2004; 57, 6: 399-406.*

19. *Borys J, Grabowska SZ, Antonowicz B, et al. Stężenie C- końcowego propeptydu prokolagenu typu I (PICP) w ocenie gojenia złamań żuchwy. Czas. Stomatol. 2004; 57, 5: 333-340.*

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The effect of Levothyroxine therapy on vitamin D and bone mineral density

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Abstract

Introduction: Treatment in thyroid function disorders, which lead to clinical hypothyroidism, is replacement with Levothyroxine. This study investigated the effect of long-term levothyroxine on vitamin D metabolism and bone mineral density in children.

Materials and Methods: Twenty-seven children with hypothyroidism receiving levothyroxine therapy, and 21 healthy controls were enrolled. Calcium, phosphorus, alkaline phosphatase, parathormone and 25 hydroxy vitamin D levels were measured from serum specimens collected from the study and control groups. DEXA scans were performed and t scores determined.

Results: A statistically significant difference was determined between the study group and control group vitamin D levels ($p<0.05$), but no statistically significant difference was determined in terms of t scores ($p>0.05$)

Discussion: Long-term therapies can affect vitamin D levels and impair bone health in later periods. Bone mineral density analysis would seem to be useful in long-term therapies.

Key Words: Levothyroxine, child, vitamin D, bone mineral density.

Introduction

Thyroid hormones affect the growth and development of the skeletal system. They exhibit these effects through osteoclastic and osteoblastic activities in the bones. However, their effects on osteoclasts are more dominant and they increase bone resorption. Thyrotoxicosis accelerates bone maturation in children in particular and may cause retardation in growth and skeletal system development (1).

Various diseases give rise to thyroid dysfunction, the clinical picture generally taking the form of hypothyroidism (2, 3). The aim in hypothyroidism treatment is to replace the deficient thyroid hormone at an appropriate dosage. Patients must be monitored at frequent intervals during thyroid hormone replacement therapy (4).

The effect on bone health and vitamin D metabolism of the levothyroxine used in treatment is the subject of debate. Studies with adults have shown that levothyroxine used in suppression doses has negative effects on bone mineral density (BMD). Some studies show that when given in a maintenance dose levothyroxine has no negative effects on bone health, while other studies have reported negative effects (5-7).

Our scan of the literature revealed a lack of sufficient information regarding thyroid hormone replacement therapy, BMD and vitamin D in children. The purpose of this study was to investigate the effects of levothyroxine on vitamin D levels and BMD in childhood.

Materials and methods

Twenty-seven children with hypothyroiditis (study group) under observation by the Atatürk University Department of Pediatric Endocrinology, Erzurum, Turkey and 21 healthy children (control group) were enrolled in the study. Ethical Committee approval was obtained before the study began. Informed consent forms were received from the parents of all participants.

Children were deemed eligible for inclusion when they were aged 6 to 18 years Patients and healthy groups have same parameters. The children that were sex and age-matched selected as controls. The children in the control group were from the same geographical area, and they were admitted to the pediatric out-patient clinic for other reasons than systemic problems. Controls were similar to

patients except for levotiroxin therapy. We viewed the records of all patients and looked at the following details: age of onset; how long drug used; laboratory parameters that included Calcium (Ca), Phophorus (P), alcaline phosphatase (ALP), Parathormone (PTH), 25OHD. All patients in the study group were selected from those regularly attending check-ups and whose growth parameters were within normal limits. Patients with hypothyroidism received levothyroxine replacement therapy for different periods at a dosage of 2-4 mg/kg.

The exclusion criteria were use of any medications known to interfere with liver or renal functions and thyroid functions, thyroid, kidney or liver disease, endocrine disorders .

Blood sample was obtained from patients after least 4 months after levotiroxine treatment started diagnosed, between 8:00 and 10:00 am after 12- h fasting. to avoid diurnal variations.

All blood samples were stored at -40 °C until analysis. In accordance with laboratory reference values, normal serum values were determined at 8.8-10.8 for Calcium (Ca) 2.8-6.0 for Phosphorus (P) and 75-400 for alkaline phosphatase (ALP).

In terms of group 25OHD levels, 15 ng/mL and below was classified as severe deficiency, 15-20 ng/mL as deficiency and values of 20 ng/mL and above as normal (8). All the tests were performed according to the manufacturer's instructions. Serum Calcium (Ca), Phosphorus (P), Alcaline Phosphatase ALP levels were determined in serum using an Roche Cobas 8000 System (Tokyo, Japan) with using Roche Diagnostics kits. 25OHD levels (ng/mL) were determined in an E-170 ECL system (Roche, Japan) with an electrochemiluminescence method. PTH (pg/mL) was measured by chemiluminescent enzyme immunoassay, IMMULITE (DPC Co, USA) autoanalyzer.

For all patients and controls T score were measured by dual energy X-ray absorptiometry (DEXA) using the (Hologic QDR 2000). The T-score is the standard deviation of the individual BMD compared to the mean BMD score of a similar sex-, age-, weight- and height-matched population.According to WHO classification, T score > –1 was considered normal, T score between –1 and –2.5 was considered osteopenia and T score = –2.5 was considered osteoporosis.

Statistical Analysis

SPSS software package version 18 (SPSS Inc., Chicago, IL) was used for statistical analysis. Data were presented as means ± standard error meaning. Oneway analysis of variance and post-hoc least significant difference (LSD) option test were used to compare between cases and controls and between the different groups. Pearson chi square test was used to analyse multiple variants. Significance was declared at p less-than- or equals slant 0.05.

Results

Twenty-seven children with hypothyroidism and a control group of 21 healthy individuals were enrolled. Mean age was 12.1 ± 0.7 in the study group and 11.8 ± 0.5 in the control group. Fourteen (51.8 %) of the study group were girls and 13 (48.1 %) boys, compared to 8 (38.1 %) girls and 13 (61.9 %) boys in the control group.

Mean length of levothyroxinee use in the study group was 7.7 ± 0.5 months (min-max, 4 - 12 months), with a mean dose of 2.7 ± 0.13 mg/kg (min-max, 2-4 mg/kg). The difference between the vitamin D levels in the study and control groups was statistically significant (Table 1).

Table 1. Comparison of the study and control group vitamin D levels

Groups	Vitamin D				P value
	Normal (n/%)	Mild (n/%)	Severe (n/%)	Total (n/%)	
Hypothyroidism	8 % 29.6	8 % 29.6	11 % 40.7	27 % 100	0.019*
Control	14 % 66.7	5 % 23.8	2 % 9.5	21 % 100	

Not The Pearson Chi square test was applied. n; number of individuals
**P≤0.05 was regarded as significant.*

Table 2. Comparison of the study and control groups in terms of t scores

Groups	T score				P value
	Normal (n/%)	Osteopenia (n/%)	Osteoporosis (n/%)	Total (n/%)	
Hypotiroidism	13 % 48.1	11 % 40.7	3 % 11.2	27 % 100	**0.251**
Control	13 % 61.9	8 % 38.1	0 % 0	21 % 100	

Note: The Pearson Chi square test was applied. No. = number of individuals. t scores > -1 were normal, while scores between (-1) and (-2.5) were regarded as osteopenia and scores <-2.5 as osteoporosis.
P≤0.05 was regarded as significant.

Table 3. Comparison of levothyroxine dosage, duration and t scores

Levothyroxine use	T score		
	Normal	Osteopenia	Osteoporosis
Dosage (mg/kg)	2.57±0.19	2.9±0.22	2.66±0.16
P value	-	0.253	0.841
duration (m)	7.53±0.63	7.36±0.6	10.3±1.66
P value	-	0.851	0.63

Note: One-way ANOVA followed by the LSD correction test were applied. t scores > -1 were normal, while scores between (-1) and (-2.5) were regarded as osteopenia and scores <-2.5 as osteoporosis. mg/kg; milligrams per kilogram; m, months.
P≤0.05 was regarded as significant.

Mean parathormone level in the study group was 39.18 ± 1.99 (min-max 26 - 67), and 38.9 ± 2.84 (min - max, 26 - 77) in the control group. The difference was not statistically significant (p = 0.935). Study and control group mean Ca, phosphorus (P) and alkaline phosphatase (ALP) values were 9.4±0.14 and 9.6±0.13; 4.1±0.19 and 5±0.23 and 399.3±22.9 and 227.3±101.1, respectively. In terms of Ca, there was no statistically significant difference between the groups (p=0.161). The differences between the groups in terms of serum P and ALP were statistically significant, with p values of p = 0.005 and p = 0.0001, respectively.

The difference between the study and control group in terms of t scores was not statistically significant (p = 0.251) (Table 2).

Data for comparison of levothyroxine dosage and z scores in the study group are given in Table 3. No statistically significant difference in intragroup levothyroxine use dosage and duration was determined.

Discussion

Thyroid hormones are associated with skeletal health. They play a role in bone formation, development, mineralization and remodeling. An excess of thyroid hormone represents a risk factor for osteoporosis by leading to acceleration in the bone remodeling process. Improvement in BMD takes place with thyrotoxicosis treatment. Hypothyroidism may also be a risk factor for fractures (9-13).

Treatment in hypothyroidism is long term. Depending on diagnosis, treatment generally starts from the neonatal period. Patients are initially monitored very closely, but as they grow older the monitoring intervals are extended. Treatment compliance problems may arise as the length of treatment grows. Even if treatments that are interrupted for short periods or used excessively as length of treatment grows are not reflected in the laboratory they can still cause long-term pathologies in systems in which thyroid hormones are involved (9, 14). Although there was no statistically significant difference in terms of BMD between subjects receiving levothyroxine therapy and the control group in this study, patients developing osteoporosis were identified in the treatment group. The effect of levothyroxine therapy on BMD is debatable. Studies have emphasized that in suppressive doses maintenance therapy that establishes a disposition to osteoporosis has no effect on BMD

(12,15-18) . In addition to subclinical or clinical hypothyroidism being a cause of secondary osteoporosis, variations in normal thyroid stimulating hormone (TSH) values can also cause alterations in BMD (9,19) . Kısakol G et al. (20) investigated Ca and bone metabolism in cases with hypothyroidism and subclinical hypothyroidism. They determined that Ca excretion was not affected in patients with subclinical hypothyroidism, but that bone turnover decreased. Experimental studies have also revealed that exogenous thyroid hormones cause a decrease in BMD. It has been reported that this effect can be prevented by reducing the dosage (21, 22) . If vitamin D deficiency is not treated it can lead to osteopenia and osteoporosis. Studies have shown low vitamin D levels in Hashimoto's thyroiditis and have emphasized that vitamin D is a factor that can lead to Hashimoto's thyroiditis through the immune system (23,24).

Vitamin D levels in the group receiving levothyroxine therapy in our study were significantly low. levothyroxine and vitamin D being metabolized in the liver may have led to this picture. Levothyroxine therapy can also increase the risk of fracture in subjects with a tendency to osteoporosis.

There are various limitations to our study. For ethical reasons, blood specimens were collected only once. Again for ethical reasons, the patient number had to be restricted. Although the study and control groups were selected from individuals with the same demographic and geographical characteristics, complete homogeneity in dietary and social conditions that might affect vitamin D levels in particular could not be established.

In conclusion, the side-effects of levothyroxine therapy need to be well known. Long-term therapies can affect vitamin D levels and impair subsequent bone health. BMD should be evaluated in long-term therapies. In cases where a decrease in BMD is identified, in the absence of any other cause, the possibility of this being due to levothyroxine therapy must be considered.

References

1. *Williams GR. Actions of thyroid hormones in bone. Endokrynol Pol. [Review]. 2009 Sep-Oct; 60(5): 380-8.*
2. *Demirkaya M, Sevinir B, Saglam H, et al. Thyroid functions in long-term survivors of pediatric Hodgkin's lymphoma treated with chemotherapy and radiotherapy. J Clin Res Pediatr Endocrinol. 2011; 3(2): 89-94.*
3. *Ergur AT, Taner Y, Ata E, et al. Neurocognitive functions in children and adolescents with subclinical hypothyroidism. J Clin Res Pediatr Endocrinol. 2012 Mar; 4(1): 21-4.*
4. *Setian NS. Hypothyroidism in children: diagnosis and treatment. J Pediatr (Rio J). [Review]. 2007 Nov; 83(5 Suppl): S209-16.*
5. *Larijani B, Gharibdoost F, Pajouhi M, et al. Effects of levothyroxine suppressive therapy on bone mineral density in premenopausal women. J Clin Pharm Ther. 2004 Feb; 29(1): 1-5.*
6. *Fujiyama K, Kiriyama T, Ito M, et al. Suppressive doses of thyroxine do not accelerate age-related bone loss in late postmenopausal women. Thyroid. 1995 Feb; 5(1): 13-7.*
7. *Marcocci C, Golia F, Bruno-Bossio G, et al. Carefully monitored levothyroxine suppressive therapy is not associated with bone loss in premenopausal women. J Clin Endocrinol Metab. 1994 Apr; 78(4): 818-23.*
8. *Misra M, Pacaud D, Petryk A, et al. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics. [Review]. 2008 Aug; 122(2): 398-417.*
9. *Wexler JA, Sharretts J. Thyroid and bone. Endocrinol Metab Clin North Am. [Review]. 2007 Sep; 36(3): 673-705.*
10. *Ozkan B. Nutritional rickets. Journal of clinical research in pediatric endocrinology 2010, 2(4): 137-143.*
11. *Kosinska A, Syrenicz A, Kosinski B, et al. Osteoporosis in thyroid diseases. Endokrynol Pol 2005; 56: 185-93.*
12. *Salama HM, El-Dayem SA, Yousef H, et al. The effects of L-thyroxin replacement therapy on bone minerals and body composition in hypothyroid children. Arch Med Sci. 2010 Jun 30; 6(3): 407-13.*
13. *Ozkan B, Doneray H, Keskin H. The Effect of Vitamin D Treatment on Serum Adiponectin Levels in Children with Vitamin D Deficiency Rickets. J Clin Res Ped Endo 2009; 1(6): 262-265*

14. *Uzzan B, Campos J, Cucherat M, et al. Effects on bone mass of long term treatment with thyroid hormones: a meta-analysis. J Clin Endocrinol Metab. 1996 Dec; 81(12): 4278-89.*

15. *Demartini Ade A, Kulak CA, Borba VC, et al. [Bone mineral density of children and adolescents with congenital hypothyroidism]. Arq Bras Endocrinol Metabol. 2007 Oct; 51(7): 1084-92.*

16. *Ribot C, Tremollieres F, Pouilles JM, Louvet JP. Bone mineral density and thyroid hormone therapy. Clin Endocrinol (Oxf). 1990 Aug; 33(2): 143-53.*

17. *Tumer L, Hasanoglu A, Cinaz P, Bideci A. Bone mineral density and metabolism in children treated with L-thyroxine. J Pediatr Endocrinol Metab. 1999 Jul-Aug; 12(4): 519-23.*

18. *Salerno M, Lettiero T, Esposito-del Puente A, et al. Effect of long-term L-thyroxine treatment on bone mineral density in young adults with congenital hypothyroidism. Eur J Endocrinol. [Clinical Trial]. 2004 Dec; 151(6): 689-94.*

19. *Harper KD, Weber TJ. Secondary osteoporosis. Diagnostic considerations. Endocrinol Metab Clin North Am. [Review]. 1998 Jun; 27(2): 325-48.*

20. *Kisakol G, Kaya A, Gonen S, Tunc R. Bone and calcium metabolism in subclinical autoimmune hyperthyroidism and hypothyroidism. Endocr J. 2003 Dec; 50(6): 657-61.*

21. *Broulik PD, Rosenkrancova J, Ruzicka P, Sedlacek R. Effects of triiodothyronine and estrogen administration on bone mass, mineral content and bone strength in male rats. Horm Metab Res. 2003 Sep; 35(9): 527-31.*

22. *Ishihara C, Kushida K, Takahashi M, et al. Effect of thyroid hormone on bone and mineral metabolism in rat: evaluation by biochemical markers. Endocr Res. 1997 Aug; 23(3): 167-80.*

23. *Stefanic M, Papic S, Suver M. Association of vitamin D gene 3'- variants with Hashimoto's thyroiditis in the Croatian population. Int J Immunogenet 2008; 35: 125- 31.*

24. *Youshiyuki Ban, Matsuo Taniyama, Yoshio Ban. Vitamin D receptor gene polymorphism in Hashimoto's thyroiditis. Thyroid 2002; 11(6): 607- 608.*

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Effects of femoral nerve block on propofol consumption and postoperative analgesia in knee arthroscopy

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Abstract

Introduction: In this study, it was aimed to investigate effects of femoral nerve block (FNB) performed before surgery on requirement of intraoperative propofol and consumption of tramadol hydrochloride used for postoperative analgesia.

Methods: After obtaining approval of Ethics Committee and informed consent, 40 adult patients (ASA I-II) undergoing elective knee arthroscopy were included to the study. Patients were randomly assigned into 2 groups by sealed envelope method. Patient-controlled analgesia (PCA) was used in Group I (n=20), whereas femoral nerve block plus PCA in Group II (n=20). Anesthesia induction was achieved by propofol and remifentanil in all patients. Rocuronium was used for muscle relaxation during induction. Anesthesia was maintained by 50% O2: Air mixture as well as intravenous propofol and remifentanil infusions. BIS values were maintained in the range of 40 to 60. Vital signs were recorded at perioperative and postoperative period.

Discussion: No significant difference was detected in propofol and remifentanil doses used between groups ($p>0.05$). Although infusion maintenance dose of propofol was lower in group II (548.52 mg) than group I (666.25 mg), difference was insignificant ($p>0.05$). When postoperative VAS scores was considered in all time points, a significant difference was found between groups ($p<0.01$). A significant difference was found in the total consumption of tramadol hydrochloride between groups ($p<0.05$). Patients in group II consumed significantly lower amounts of tramadol hydrochloride when compared to those in group I (158.07 mg vs. 197.66 mg, respectively).

Conclusion: According to these results, we think that preemptive FNB is insufficient to reduce anesthetic consumption induced by surgical stimulation; however, it is effective in analgesia in early period after surgery in knee arthroscopy.

Key words: Tramadol, propofol, femoral nerve, arthroscopy, analgesia.

Introduction

Outpatient procedures are preferred due to decreased length of hospital stay, cost and risk of hospital acquired infections (1-3). Knee arthroscopy is one of the most common outpatient procedures. Thus, short-acting anesthetic agents should be selected for early ambulation in knee arthroscopies. In addition, postoperative pain, nausea and vomiting have to be effectively managed (4-7). It has been shown that preoperative nerve blocks or use of analgesics may prevent central hyperexcitability induced by surgical trauma. However, since peripheral and central sensitization will continue as stimuli arising from surgical areas continue, postoperative analgesia should be balanced in association to preemptive analgesia (8-10). In the present study, it was aimed to investigate effects of femoral nerve block (FNB) performed before surgery on requirement of intraoperative propofol and consumption of tramadol hydrochloride used for postoperative analgesia.

Patients and Methods

After obtaining approval of Ethics Committee and informed consent, it was planned to recruit 40 adult patients (ASA I-II) who were scheduled for elective knee arthroscopy. Patients with neuromuscular disorder, liver and kidney dysfunction, previous history of opiate use, peripheral neuropathy and allergy to drugs evaluated were excluded. Preoperative assessments were performed

on the day before surgery; and verbal and written consents were obtained after informed about study. In addition, information about visual analog scale (VAS) and patient-controlled analgesia method were reminded to all patients before surgery. After sedation with 0.03 mg/kg IV midazolam given 30 minutes before operation, patients were transported to operation room; standard monitoring with heart rate (HR), non-invasive blood pressure (NIBP), peripheral oxygen saturation ($Sp0_2$) and bispectral index (BIS) was initiated and baseline values were recorded (Infinity® Kappa Patient Monitor-Draeger, Germany). Patients were randomly assigned into 2 groups by sealed envelope method. Patient-controlled analgesia (PCA) was used in Group I (n=20), whereas femoral nerve block plus PCA in Group II (n=20). In both groups, 50 mg tramadol hydrochloride was administered 15 minutes before the end of operation according to PCA (Bodyguard – 575, Pain Manager) protocol. PCA settings were as follows: bolus dose, 20 mg; lock-out interval, 15 minutes; 4 hours limit, 200 mg; no basal infusion. In the group II, ultrasound-guided (Vivid e, GE Healthcare) femoral nerve block was performed via inguinal paravascular technique 30 minutes before surgery. After achieving aseptic and antiseptic conditions, femoral nerve was localized via peripheral nerve stimulatory (Stimuplex HNS12, Braun) using a 22G/50 mm, peripheral nerve stimulatory needle under (Stimuplex D,Braun, Germany) ultrasound guidance, while patient was in the supine position. Thirty ml 0.25% levobupivacaine was given with negative aspiration in every 5 ml in the presence of ongoing contractions in quadriceps femoris muscle at 0.5 mA. Diffusion of local anesthetic around nerve was observed on sonography. Efficiency of femoral block was confirmed by decreased sensation at anterior areas of thigh region and occurrence of motor block in quadriceps femoris muscle. In all patients, standard anesthesia technique was used. Anesthesia induction was achieved by propofol (2 mg/kg) and remifentanil (1 µg/kg/min). Rocuronium (0.06 mg/kg) was used for muscle relaxation during induction. Maintenance anesthesia was initiated by propofol (100 µg/kg/min), remifentanil (0.1 µg/kg/min) and 50% O2: air mixture. Mechanical ventilation, respiratory volume and frequency were performed as maintaining end-tidal carbon dioxide ($PETCO_2$) between 30 to 35 mmHg. BIS values were maintained in the range of 40 to 60 during anesthesia in patients in which BIS monitoring (Infinity BISx SmartPod-Draeger, Germany) was performed. When BIS value increased above 60, 10 µ/kg/min increments of propofol concentration was made, while decrements of 10 µ/kg/min was made in every 5 minutes when BIS value decreased below 40. During skin suturing, propofol and remifentanil infusions were discontinued. Tourniquet was used in all cases to achieve better surgical exposure. PCA protocol was initiated at postoperative period in all patients. Pain was assessed by blinded pain staff via VAS (0: no pain; 10: worst pain experienced). It was planned to administer an additional tramadol hydrochloride bolus of 20 mg in patients with VAS score>4. At perioperative period, HR, NIBP, SpO_2 and BIS values at baseline (after sedation), before intubation after anesthesia induction and on minutes 1, 10, 30 and 60 after intubation were recorded. At postoperative period, pain scores as well as HR, NIBP, respiration rate, sedation score (1: anxious, agitated; 2: calm, cooperative; 3: response to verbal stimulus; 4: response to physical stimulus; 5: response to painful stimulus; 6: no response to painful stimuli) at hours 0.5, 1, 2, 4 and 6 and postoperative nausea and vomiting were recorded. Bradycardia was defined as HR<50, while decrease of mean arterial pressure (MAP) greater than 30% of baseline as hypotension and respiration rate below 8/min as respiratory depression. It was planned to administer 10 mg IV metoclopramide in the presence of severe nausea-vomiting; 0.1 mg IV naloxone (every 2-3 minutes until response was achieved) in the presence of respiratory depression; 0.5 mg IV atropine in case of bradycardia; and 500 mL rapid crystalloid infusion, if no response, 10 mg IV ephedrine in case of hypotension. As surgical procedures were performed in outpatient basis and PCA protocol cannot be administered at out-of-hospital settings, postoperative pain assessments were limited with 6 hours. PCA protocol was continued in patients staying in hospital in subsequent hours, while 500 mg PO paracetamol (q8 hours) was recommended to those discharged.

Statistical Analysis

Statistical analyses were performed by using SPSS version 13.0 (Statistical Package for Social Sciences). The power of this study is above 90% according to power calculations. ANOVA test was used in repeated measurements, while paired sample t test to compare changes over time. Results were expressed as mean ± SE. $p<0.05$ was considered as significant.

Results

Overall, 40 patients were included to the study. No significant difference was detected in age, height, weight, gender, duration of operation and duration of anesthesia between groups (Table 1) ($p>0.05$). No significant difference was found between groups regarding induction dose of propofol, infusion maintenance dose of propofol, induction dose of remifentanil and infusion maintenance dose of remifentanil (Table 2) ($P>0.05$). Although infusion maintenance dose of propofol was lower in group II (548.52 mg) than group I (666.25), difference was insignificant (Table 2) ($p=0.150$). Figure 1 presents VAS scores on hours 0.5, 1, 2, 4 and 6 after surgery. When all time points were compared, a significant difference was found between groups ($p=0.007$, $p<0.01$). A significant difference was found in the consumption of tramadol hydrochloride between groups. Patients in group II (158.07) consumed significantly lower amounts of tramadol hydrochloride when compared to those in group I (197.66 mg) (Figure 2; $p=0.021$, $p<0.05$). When HR ($p=0.147$), MAP ($p=0.191$) and BIS values ($p=0.068$) at baseline, before intubation, after intubation and on minutes 1, 10, 30 and 60 after surgery were compared between groups, no significant difference was detected. No significant difference was found in HR ($p=0.930$), MAP ($p=0.147$) and sedation ($p=1.0$) values on hours 0.5, 1, 2, 4 and 6 after surgery among groups. In Group 1, there was nausea in 3 patients and vomiting in 1 patient at postoperative period, while there was nausea in 4 patients, but there was no patient with vomiting. In these patients, 10 mg IV metochlopramide was used. No hypotension, bradycardia or respiratory depression requiring treatment was observed in both groups.

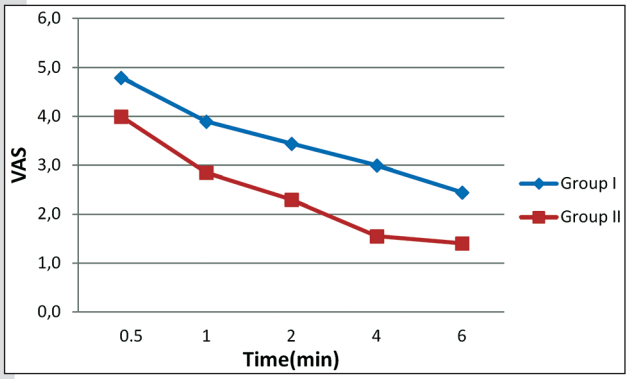

Figure 1. Postoperative VAS scores was statistically lower in group II than group I ($p< 0.01$).

Table 1. Demographic data (Mean±SD)

	Group I (n=20)	Group II (n=20)	P
Age (yr)	37.4 ± 12.3	36.7 ± 12.8	0.872
Weight (kg)	78.4 ± 11.6	78.0 ± 13.9	0.912
Height (cm)	171.9 ± 9.3	168.1 ± 6.2	0.139
Gender (F/M)	7/13	8/12	
Duration of surgery (min)	71.2 ± 26.6	70.2 ± 26.6	0.911
Duration of anaesthesia (min)	85.9 ± 28.5	85.9 ± 30.1	1.0

p> 0.05; F: Females; M: Males; Grup I: PCA group; GroupII: Femoral nerve block+PCA group

Table 2. Groups consumed doses of propofol and remifentanil (Mean±SD)

	Group I (n=20)	Group II (n=20)	P
Induction dose of Propofol (mg)	156.6 ± 23.2	156.0 ± 27.9	0.912
Induction dose of Remifentanil (µg)	78.4 ± 11.6	78.0 ± 13.9	0.912
Maintenance dose of Propofol (mg)	666.2 ± 285.9	548.5 ± 216.4	0.150
Maintenance dose of Remifentanil (µg)	576.9 ± 232.5	553.1 ± 240.3	0.752

p> 0.05; Group I: PCA group; Group II: Femoral nerve block+PCA group

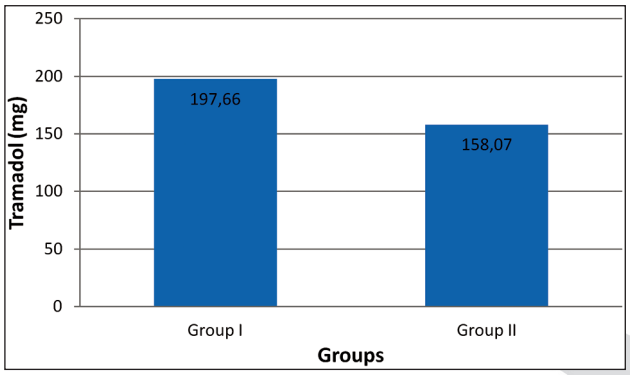

Figure 2. Patients in group II consumed significantly lower amounts of tramadol hydrochloride when compared to those in group I ($p< 0.05$)

Discussion

Postoperative pain is one of the most commonly encountered problems in outpatient procedures. Sufficient postoperative analgesia facilitates and accelerated early rehabilitation (11, 12). It is now known that development of postoperative pain can be dramatically controlled by providing sufficient preoperative pain control. To this end, it is aimed to block nosiseptive impulses before surgical intervention by using analgesic methods at preoperative or perioperative and to achieve analgesia at intraoperative and postoperative period (8-10). In the present study, we performed a femoral nerve block before surgery in knee arthroscopy in outpatient basis. We assessed requirement of intraoperative propofol, postoperative pain levels and postoperative tramadol hydrochloride consumption in these patients. We considered that anesthesia depth was similar between groups in this study, as there was no significant difference in hemodynamic parameters and BIS values during intraoperative period. Intraoperative propofol consumption was decreased in the group received femoral nerve block when compared to the group which did not. However, this reduction failed to reach statistical significance. Presumably, pneumatic tourniquet used at thigh level and sensorial innervations of posterior areas of thigh and knee region by sciatic nerve and its branches prevented further decrease in propofol consumption by causing intraoperative nosiseptive impulses arising from these regions. However, reduction in the consumption of propofol may be caused by sensorial block at anterior areas of thigh and knee region resulted from preemptive femoral block. On the other hand, somewhat decrease in propofol consumption in the FNB group may be important in outpatient patients, despite it was statistically insignificance. Advantage of early ambulation which can be achieved by lower amounts of anesthetic agent should not be ignored. Although there are reports suggesting that FNB has no effect on postoperative analgesia in patients undergoing knee surgery, it is usually considered as effective in literature (13-18). In a study on patients underwent anterior cruciate ligament reconstruction, Edkin et al. proposed that 92% of the in-patients who received a single dose FNB didn't need parenteral opiates within first 24 hours after surgery (16). In agreement to our study, Peng et al. reported that FNB decreased pain score and opiate consumption within first 18 hours after surgery (17). In the studies, FNB provided better analgesic efficiency when compared to opiates administered by PCA and NSAIDs in patients underwent arthroscopic knee surgery (18, 19). In a study by Rosaeg et al. in which preemptive efficiency of multimodal analgesia regimen consisting from NSAIDs, intraarticular injection and FNB was evaluated, authors applied this regimen 15 minutes before and after skin closure and reported that pain score and opiate consumption at recovery room was decreased by multimodal analgesia regimen (20). In our study, we performed FNB before surgical intervention in order to take advantage of preemptive analgesia. Postoperative pain should be adequately relieved at early period to achieve success in outpatient procedures. In addition, it has been reported that preemptive analgesia techniques have a role not only in the control of postoperative pain but also in the prevention of neuropathic pain which may continue weeks to months after surgery (21, 22). Mulroy et al. reported that FNB added to multimodal analgesic regimen which consisted of NSAI drug and cold application provided better analgesia in postoperative period in patients underwent arthroscopic knee surgery (23). Again, Williams et al. reported that FNB by a single injection as a part of multimodal treatment helps early postoperative analgesia in patients undergoing outpatient procedures (24). In agreement to above-mentioned studies, tramadol hydrochloride consumption was decreased and lower pain scores were achieved by preemptive

FNB used as a part of multimodal analgesia regimen in the present study. We think that it is important to reduce opiate dose in outpatients who will be discharged to home on the same day, since parenteral opiate use and PCA protocol can be problematic at home. After knee surgery, intraarticular local anesthetic injections are also widely used as a part of multimodal analgesia regimen. However, in a study by İskandar et al., it was reported that FNB provided better analgesia in addition to decreased morphine consumption, vomiting and incidence of sedation in arthroscopic knee surgeries (11). In outpatient procedures, the second most important problem is postoperative nausea and vomiting (PONV). Several factors including anesthesia technique, type of surgery, opiate use, age and gender affects PONV incidence (6, 25, 26). In the literature, it has been reported that selection of total intravenous anesthesia (TIVA) rather than inhalation anesthesia and using propofol as hypnotic agent dramatically decrease incidence of nausea and vomiting (27). In our study, incidence of postoperative nausea and vomiting was low in all patients. We think that anti-emetic effect of propofol used in TIVA in both groups contributed to this finding.

In conclusion, there was a reduction in propofol consumption during intraoperative period in group received femoral nerve block. However, this reduction failed to reach statistical significance. Nonetheless, a significant reduction was observed in tramadol hydrochloride consumption and VAS scores at postoperative period. According to these results, we think that preemptive femoral nerve block is insufficient to reduce anesthetic consumption induced by surgical stimulation; however, it is effective in analgesia in early period after surgery in knee arthroscopy.

References

1. *Goranson BD, Lang S, Cassidy JD, et al. A comparison of three regional anaesthesia techniques for outpatient knee arthroscopy. Can J Anaesth 1997; 44: 371–376.*
2. *Akçalı DT, Coşkun D, Çelebi H. [Principles of outpatient anesthesia]. Anestezi Dergisi 2009; 17: 117–132.*
3. *White PF. Ambulatory anesthesia advances into the new millennium. Anesth Analg. 2000; 90: 1234-1235.*
4. *Rawal N, Hylander J, Nydahl PA, et al. Survey of postoperative analgesia following ambulatory surgery. Acta Anaesthesiol Scand. 1997; 41: 1017-1022.*
5. *White PF, Eng M. Fast-track anesthetic techniques for ambulatory surgery. Anaesthesiol. 2007; 20: 545-557.*
6. *Carroll NV, Miederhoff P, Cox FM, Hirsch JD. Postoperative nausea and vomiting after discharge from outpatient surgery centers. Anesth Analg. 1995; 80: 903-909.*
7. *Awad IT, Chung F. Factors affecting recovery and discharge following ambulatory surgery. Can J Anaesth. 2006; 53: 858-872.*
8. *Wall PD: The preventation of postoperative pain. Pain 1988; 33: 289-290.*
9. *Dahl JB, Moiniche S. Pre-emptiveanalgesia. Br Med Bull. 2004; 13: 13-27.*
10. *Dahl JB, Kehlet H. The value of pre-emptive analgesia in the treatment of postoperative pain. Br J Anaesth 1993; 70: 434-439.*
11. *Iskandar H, Benard A, Ruel-Raymond J, et al. Femoral block provides superior analgesia compared with intra-articular ropivacaine after anterior cruciate ligament reconstruction. Reg Anesth Pain Med. 2003; 28: 29-32.*
12. *Chung F, Ritchie E, Su J. Postoperative pain in ambulatory surgery. Anesth Analg. 1997; 85: 808-816.*
13. *Macalou D, Trueck S, Meuret P, et al. Postoperative analgesia after total knee replacement: the effect of an obturator nerve block added to the femoral 3-in-1 nerve block. Anesth Analg 2004; 99: 251–254*
14. *Schwarz S, Franciosi L, Ries CR, et al. Addition of femoral 3-in-1 blockade to intraarticular ropivacaine 0.2% does not reduce analgesic requirements following arthroscopic knee surgery. Can J Anaesth 1999; 46: 741-747.*

15. *Tierney E, Lewis G, Hurting MB, Johnson D. Femoral nerve block with bupivacaine 0.25 per cent for postoperative analgesia after open knee surgery. Can J Anaesth 1987; 34: 455-458.*

16. *Edkin BS, Spindler KP, Flanagan JF. Femoral nerve block as an alternative to parenteral narcotics for pain control after anterior cruciate ligament reconstruction. Arthroscopy 1995; 11: 404-409.*

17. *Peng P, Claxton A, Chung F, et al. Femoral nerve block and ketorolac in patients undergoing anterior cruciate ligament reconstruction. Can J Anaesth 1999; 46: 919-924.*

18. *Matheny LM, Hanks GA, Rung GW, et al. A comparison of patient controlled analgesia and continuous lumbar plexus block after anterior cruciate ligament reconstruction. Arthroscopy 1993; 9: 87-90.*

19. *Frost S, Grossfeld S, Kirkley A, et al. The efficacy of femoral nerve block in pain reduction for outpatient hamstring anterior cruciate ligament reconstruction: a double-blind, prospective, randomized trial. Arthroscopy 2000; 16: 243-248.*

20. *Rosaeg OP, Krepski B, Cicutti N, et al. Effect of preemptive multimodal analgesia for arthroscopic knee ligament repair. Reg Anesth Pain Med. 2001; 26: 125-130.*

21. *Katz J, Kavanagh BP, Sandler AN, et al. Preemptif analgesia. Clinical evidence of neuroplasticity contributing to postoperative pain. Anesthesiology 1992; 77: 439-446.*

22. *Woolf CJ, Chong MS: Preemptive analgesia treating postoperative pain by preventing the establishment of central sensitization. Anest Analg 1993; 77: 362-379.*

23. *Mulroy MF, Larkin KL, Batra MS, et al. Femoral nerve block with 0.25% or 0.5% bupivacaine improves postoperative analgesia following outpatient arthroscopic anterior cruciate ligament repair. Reg Anesth Pain Med. 2001; 26: 24-29.*

24. *Williams BA, DeRiso BM, Figallo CM, et al. Benchmarking the perioperative process: III. Effects of regional anesthesia clinical pathway techniques on process efficiency and recovery profiles in ambulatory orthopedic surgery. J Clin Anesth 1998; 10: 570-578.*

25. *Watcha MF, White PF. Postoperative nausea and vomiting. Its etiology, treatment, and prevention. Anesthesiology. 1992; 77: 162-184.*

26. *Cohen MM, Duncan PG, DeBoer DP, Tweed WA. The postoperative interview: assessing risk factors for nausea and vomiting. Anesth Analg. 1994; 78: 7-16.*

27. *Gupta A. Evidence-based medicine in day surgery. Curr Opin Anaesthesiol 2007; 20: 520-525.*

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Immuno-compromised patients resistance to antibiotic and plant extract

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Abstract

Background: Patients with immuno-compromised diseases as cancer were subjects to infections as a result of several factors as obstruction or constriction of airways or ducts, erosion of tumour, and alternation of host defences secondary to infiltration of bone marrow.

Objective: This study was carried on 50 cancer patients admitted to King Abdulaziz University Hospital. They include males and females and their ages ranged from 36 to 75 years. Our study aims to evaluate the activity of some antimicrobial agents against some clinical bacterial isolates isolated from cancer patients.

Material and Methods: Sputum and urine samples were collected from infected cancer patients, culture was performed and isolates were identified. These isolates were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Psudomonas aeruginosa*. These isolates were tested against 18 antibiotics.

Results: The most effective antibiotics were imipenem which most of all isolates showed susceptible to imipenem, also most of strains showed resistance for penicillin and some of strains showed intermediate to claforan. All isolates were tested in vitro against 20 essential oils from commercial origin, most strains showed susceptible to thyme oil and were multi-resistance against for garlic oil and guava oil. *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates showed highest minimum inhibitory concentration with imipenem antibiotic and also with thyme oil. Combination between imipenem antibiotic and thyme oil showed greater effect than that of each agent alone.

Key words: Immuno-compromised, Antibiotics, Plants extract.

Introduction

Infection has been recognized as one of the major obstacles to the successful management of patients with malignant tumours. Aerobic and anaerobic bacteria source are a major cause of infection in necrotic tumours, especially when they occur in proximity to site where these bacteria resides as part of the normal flora (1). Although surgical removal or evacuation of the purulent fluid is preferred, this is not always feasible in patient with malignant tumour. Antimicrobial therapy against potential bacteria pathogens is often the sole therapy or is used along with surgical drainage or removal of the infected area (2, 3).

Patients with cancer are subjects to infections as a result of several factors notably obstruction or constriction of airways or ducts, erosion of tumour involving the protective integument or mucosa, alternation of host defences secondary to infiltration of bone marrow, reduced or altered immunoglobulin or cytokines production or as results of chemotherapy (4). Specific infecting organisms may be predicted based on the specific defect in host defences. For example, patient with myeloma or lymphocytic leukaemia may develop infections with encapsulated bacteria as a result of decreased B-lymphocyte numbers or function, and those with lymphomas may incur a variety of intracellular bacterial, fungal, and viral infections as a result of decreased T-lymphocyte function. Neutropenia is the most frequently encountered host cell defect in patients with cancer and predicts the development of bacteremia caused by Gram-positive and Gram-negative bacteria (5).

Recent changes in microbial ecology and antimicrobial resistance profiles have highlighted the need for continued revaluation of antimicrobial therapy in these patients (6). Several new antibi-

otics with enhanced activity against organisms frequently isolated from patients with cancer have been introduced and studied recently (7).

The International Antimicrobial Therapy Cooperative Group of the EORTC has studied meropenem alone, piperacillin tazobactam, and ceftriaxone, each with single daily dose amikacin: these regimens compare favorably with ceftazidime plus amikacin for empirical treatment of fever in neutropenic patients (8). Preventive measures are important understudied and the optimal antibiotic approaches to prophylaxis remains unclear. Adjunctive measures prophylactic and therapeutic colony stimulating factors play an important role (9,10).

Infection is the most common complication of chemotherapy-induced neutropenia. Bacterial infections predominant during early stages of a neutropenic episode, where as invasive fungal infection tend to occur later. The increasing rates of antimicrobial resistance among both Gram-positive and Gram negative pathogens from patients with neutropenia are posing new challenge (11). These challenges are compounded by the fact that relatively few new drugs are being developed (12) particularly those that treat resistant Gram-negative organisms. MRSA strains frequently demonstrate multiple drug resistance to many antimicrobial agents. Difference in resistance rate may be explainable by a higher spontaneous mutation rate Master development of resistance in methicillin resistant S. aureus (13).

The major mechanism of resistance to ß Betalactam antibiotics is 3-lactamase production. Both plasmids mediated and chromosomally mediated ß-lactamase production can occur (14).

The essential oil of Thymus vulgaris showed a wide antibacterial activity against microorganisms that had developed resistance to antibiotics as MRSA and vancomycin resistant Enterococcus faecium (15,16). Aim of the work: to evaluate the activity of some antimicrobial agents against some clinical bacterial isolates isolated from cancer patients. The infection of cancer by antibiotic resistance bacteria are an important and serious problem in clinical field.

Materials and methods

This study was carried on 50 cases of cancer patients of King Abdulaziz University, 31 males and 19 females, their ages ranged from 36 - 75 years old; they were 31 males and 19 females. All sputum and urine samples were collected from lung and urinary bladder cancer patients. All the previous cases were subjected to the following;

1 Cultivation: different samples were cultured on CLED agar, Blood agar, MacConkey agar and Nutrient agar. Isolation and purification were earned out (17).

2 Identification of bacterial isolates: conventional methods for the identification and characterization of isolates were employed (18,17) , including gram staining, biochemical reactions.

3- Antibiotic susceptibility testing: -All bacterial strains isolated were tested for their antimicrobial sensitivity by standardized disc diffusion technique (19,20).

The isolates were tested against 18 antibiotics namely; Imipenem, Cefatoxime, Gentamicin, Velosef, Chloramphinicol, Rifampicin, Streptomycin, Tetracycline, Flumox, Penicillin, Vancomycin, Amoxicillin, Oxacillin, Negram, Dursif, Sxt, Erthromycin, Ampicillin.

4 Determination of the minimum inhibition concentrations (MICs) and Minimum bactericidal concentration (MBCs) of one antibiotic against selected isolates:

- The experimented bacteria were treated separately with different concentration of imipenem antibiotic under test in nutrient broth and in solid agar and the MICs and MBCs were determined.
- For detection of MICs: In Wassermann tubes two fold serial dilutions of the antibiotics were made from the diluted stock solution using broth is diluted, 10 tubes each contain 0.5ml nutrient broth containing tested organism was added .All the tubes were incubated at 37ºC for 24 hours and examined for turbidity as indicator f or bacterial growth (19).
- For detection of MBCs: One hundred µ1 was taken from each MIC concentration as well as the lower concentration introduced into nutrient agar. The plates were incubated at 37 ºC for 24 hours (19).

5 Effect of essential oils on the growth of isolates: -Effect of essential oils earned out for fifty isolates by disc diffusion test. The

isolates were tested against 20 volatile oils of (Carawaya, Origanum, Pepemit, Spanish Lemon, Clove, Anise, Cinammen, Lavender, Guava, Cucalyptus, Jasmine, fol, Rosemary, Shish, Wheat germial, Fennel, Chamommil, Garlic, Ricinus Communis, Thyme). The antibacterial activity of twenty volatile oils was done by disc diffusion method and determined as inhibition zone in mm.

The antibacterial activity of twenty essential oils was tested by filter paper disc diffusion method. Muller – Hinton agar was used. Sterile 5 mm discs saturated with 10 µ of tested oil were placed on the surface of agar plates. The plates were then incubated for 24 hours at 37 ºC. The results were recorded by measuring the zones of growth inhibition surrounding the discs (21).

6 Determination of bacteriostatic and bactericidal concentration of thyme oil were carried out by serial dilution. In the study 50 ul of bacteria suspension adjusted to Macfarland was added to 2ml broth containing 1 ml oil in glass test tubes and make 10 serial dilutions. All tubes were incubated for 24 hours at 37 ºC (21).

7 Effect of combination of Imipenem antibiotic and Thyme oil on the growth of bacteria.

The combination mixture was consisting of thyme oil with concentration (10 ug) and imipenem antibiotic with concentration (10 ug) against selected strains. Few colonies of tested organisms were emulsified in small volum of sterile nutrient broth and incubated for 24 hours at 37 ºC, the inhibition zones were measured in mm (21).

Results

Table 1. The percentage of bacterial species isolates from the patients

Bacterial species	Total isolates	Percentage %
S. aureus	18	36%
E. coli	15	30%
K. pneumoniae	13	26%
P. aeruginosa	4	8%
Total	50	100%

Table 2. Percentage of susceptibility (S), intermediate (I) and resistance (R) of S.aureus, E. coli, P. aeurginosa , K.pneomonaie against antibiotics

Bacterial Isolates and Antibiotics	S. aureus			E. coli			K .pneumonaie			1 P. aeruginosa		
	S	I	R	S	I	R	S	I	R	S	I	R
	%	%	%	%	%	%	%	%	%	%	%	%
1PM	100	0	0	93.3	6.6	0	76.9	0	23	100	0	0
CTX	33.3	28	38.8	13.3	53.3	33.3	15.3	38.4	46.1	0	0	100
GN or CN	67	5.5	27.7	20	6.6	73.3	15.3	23	61.5	0	0	100
CE	44.4	22.2	33.3	6.6	0	93.3	23	7.6	69.2	0	0	100
C	55.5	11.1	33.3	40	6.6	53.3	23	0	76.9	0	0	100
RA	83.3	5.5	11.1	0	0	100	23	0	76.9	0	0	100
S	44.4	16.6	38.8	20	26.6	53.3	30.7	0	69.2	0	25	75
TE	33.3	11.1	55.5	6.6	20	73.3	15.3	7.6	76.9	0	0	100
AFor FL	55.5	11.1	33.3	0	0	100	7.6	7.6	84.6	0	0	100
P	38.8	0	61.1	0	0	100	15.3	0	84.6	0	0	100
VA	50	5.5	44.4	13.3	0	86.6	15.3	0	84.6	0	0	100
Amx	16.6	11.1	72.2	13.3	0	86.6	23	0	76.9	0	0	100
OX	22.2	0	77.7	6.6	0	93.3	7.6	0	92.3	0	0	100
NA	33.3	11.1	55.5	60	0	40	38.4	0	61.5	25	0	75
CFR	16.6	16.6	66.6	6.6	6.6	86.6	7.6	0	92.3	0	0	100
SXT	27.7	0	72.2	33.3	6.6	60	30.7	0	69.2	25	0	75
E	16.6	11.1	72.2	0	6.6	93.3	15.3	0	84.6	0	0	100
Amp	16.6	0	83.3	33.3	6.6	60	15.3	0	84.6	0	0	100

Table (1) showed that the common pathogenic isolates isolated from patients were identified as S. aureus, E. coli, K. pneumoniae and P. aeruginosa. The hightest percentage was obtained by S. aureus. This table revealed that S. aureus was the prevalent isolated organism (36%).

Table (2) showed the antimicrobial activity of different types of antibiotics against all strains recovered from different clinical specimens. From this table ,it reveled that the most effective antibiotic against all strains was Imipenem.

Table (3) showed that, the activity of different types of volatile oils against all isolates recovered from different clinical specimens. The data demonstrated in table (3 and 4) revealed that ,the oils showed a wide variation in their antibacterial activity against Gram positive as S. *arueus* and Gram negative as *E. coli, K. . pneumonia* and *P. aeruginosa.* The result found that, the most effective volatile oils against all isolates was thymol oil.

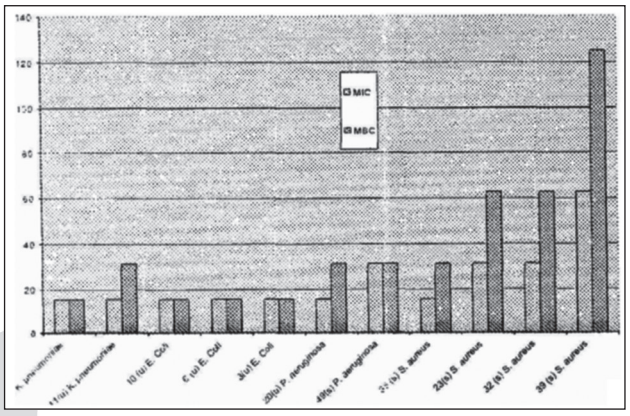

Figure 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of imipenem antibiotic for resiatant isolated microorganisms

Table 3. Effect of crude volatile oils on the growth of Gram positive bacterial isolates. (Inhibition zone in mm)

Volatile oils Bacterial isolates	Caraway	Origanum	Peppermint (O) a.	Lemon	Clove	Anise	Cinnamon	Lavander	Guava	Edcatyptus	Jasmine	Folia	Roseman	Santonica	Wheat germinal	Fennel	Chamomil	Garlic	Ricinus communis	Thyme
22 S. aureus	13	0	2	0	7	0	8	11	4	0	11	0	0	0	0	0	0	0	0	24
23s S. aureus	11	0	0	0	6	0	0	4	0	0	8	0	0	0	0	0	0	0	0	10
24 S. aureus	0	0	0	0	6	0	9	9	0	0	8	0	0	0	0	0	0	0	0	9
25s S. aureus	8	0	6	12	9	1	7	9	0	6	0	0	0	0	0	0	0	0	0	0
26s S. aureus	9	4	7	13	8	7	12	7	0	0	0	0	0	0	0	0	0	0	0	27
27s S. aureus	6	0	7	0	13	0	12	10	0	4	6	6	6	8	0	0	0	0	0	24
28s S. aureus	5	0	6	4	6	0	4	9	0	4	0	0	0	0	0	0	0	0	0	12
29s S. aureus	6	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
30s S. aureus	14	0	0	0	5	9	16	6	12	0	12	6	0	0	0	0	0	0	0	27
31s S. aureus	5	0	0	0	10	0	8	7	0	0	8	0	0	0	0	0	0	0	0	9
32s S. aureus	12	0	14	19	22	12	9	7	0	4	5	0	0	0	0	0	0	0	0	19
33s S. aureus	6	0	2	0	14	0	12	9	6	0	9	0	0	0	0	0	0	0	0	19
34s S. aureus	2	0	0	0	0	0	7	0	0	0	1	0	0	0	0	0	0	0	0	8
35s S. aureus	9	0	9	12	16	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0
36s S. aureus	6	0	0	7	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	16
37s S. aureus	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38s S. aureus	9	0	0	6	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	7
39s S. aureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4

Table 4. Effect of crud volatile oils on growth of Gram negative bacterial isolates(Inhibition zone mm)

Volatile oils And Bacterial isolates	Caraway	Origanum	Peppermint (O) a.	Lemon	Clove	Anise	Cinnamon	Lavander	Guava	Edcatyptus	Jasmine	Folia	Roseman	Santonica	Wheat germinal	Fennel	Chamomil	Garlic	Ricinus communis	Thyme
1μ E. coli	6	0	5	7	8	3	4	13	0	0	4	7	0	0	0	0	0	0	6	0
2μ E. coli	2	0	0	7	0	0	6	0	0	0	9	6	0	0	0	0	0	0	0	7
3μ E. coli	4	0	3	5	13	2	5	11	0	0	4	0	0	0	0	0	0	0	0	7
4μ E. coli	9	0	2	8	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
5μ E. coli	0	4	2	2	3	4	5	6	6	0	8	6	4	4	5	0	0	0	0	14
6μ E. coli	5	0	4	0	0	0	11	11	0	0	9	9	0	0	0	0	0	0	0	21
7μ E. coli	4	3	6	0	0	0	12	7	0	0	0	0	0	0	0	0	0	7	0	9
8μ E. coli	0	0	0	0	5	0	8	6	0	0	4	9	0	0	0	0	0	0	0	13
9μ E. coli	3	0	0	9	11	2	3	9	0	5	5	4	7	0	0	0	0	8	0	8
10μ E. coli	11	0	4	0	0	0	11	0	0	0	9	8	0	0	0	0	0	0	0	16
21sμ E. coli	5	0	0	9	6	0	5	8	0	4	5	5	0	0	0	0	0	0	0	9
40sμ E. coli	5	0	4	0	14	10	12	9	0	0	1	0	0	0	0	0	0	0	0	0
41sμ E. coli	9	7	16	14	9	14	0	0	0	0	0	0	0	0	0	0	0	0	0	11
42sμ E. coli	8	0	7	11	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	13
43sμ E. coli	7	0	14	9	11	12	0	7	0	0	7	8	0	0	0	0	0	0	0	12
11μ K.pneumonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
12μ K.pneumonia	5	0	5	12	13	0	4	3	0	11	0	0	0	0	0	0	0	0	0	10
13μ K.pneumonia	0	0	0	0	5	0	0	11	0	0	13	7	0	0	0	0	0	0		19
14μ K.pneumonia	0	0	0	0	15	0	6	11	0	0	11	8	0	0	0	25	0	0	0	24
15μ K.pneumonia	0	0	0	0	15	0	8	9	0	0	11	10	0	0	0	0	0	0	0	17
16μ K.pneumonia	0	0	0	0	0	0	0	0	0	0	8	7	0	7	0	0	0	0	0	33
17μ K.pneumonia	6	0	0	0	0	0	7	0	0	0	3	0	0	0	0	0	0	0	0	7
18μ K.pneumonia	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	8
19μ K.pneumonia	9	0	10	11	4	0	13	0	0	0	9	4	0	0	0	0	0	0	0	11
44sμ K.pneumonia	4	0	5	6	7	0	4	6	0	9	0	0	0	0	0	0	0	0	0	8
45sμ K.pneumonia	10	5	7	11	4	4	14	5	0	0	6	7	0	0	0	0	0	0	0	14
46sμ K.pneumonia	10	4	0	12	7	3	0	14	0	0	9	6	0	0	0	0	0	0	0	34
47sμ K.pneumonia	8	0	0	12	0	0	9	0	0	0	8	7	0	0	0	0	0	0	0	19
20μ P.aeruginosa	0	0	2	6	0	0	9	0	0	0	6	5	0	0	0	0	0	0	0	17
48sμ P.aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49sμ P.aeruginosa	6	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	7
50sμ P.aeruginosa	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0

Table 5. Pr oduction of B-lactamase enzyme by multi resistant isolates.

Bacterial isolates	B-lactamase production (Optical density)
S. aureus 23 (s)	1.33
S. aureus 38(s)	1.35
S. aureus 33 (s)	1.32
K. pneumoniae 1 l(u)	1.02
K. pneumoniae 16(u)	1.05
K. pneumoniae 17(u)	1.08
E. coli 5(u)	0.95
E. coli 6(u)	0.97
E. coli 1 0(u)	0.98
P. aeruginosa 49(s)	0.33
P. aeruginosa 50 (s)	0.35

Table (5) showed that, the production of ß-lactamase enzyme by multir esistant isolates The production of ß- lactamase was obtained by all isolates. The highest production of ß-lactamase was by S. aureus followed by K. pneumonia and E. coli where the least production was obtained by P. aeruginosa isolates.

Figure (1) showed that, the MIC arid MBC of the four selected isolates against imipenem antibiotic It has been found that S. aureus no (32) showed the highest MIC and MBC, it was 62.5 ug and 125 ug respectively.

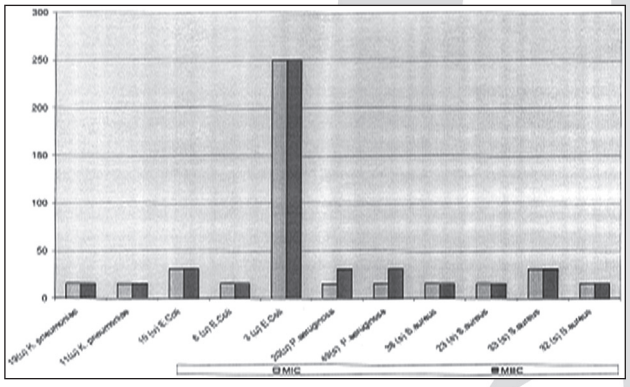

Figure 2. The Bacteriostatic and Bactericidal effect of thyme oil on tested isolates.

Figure (2) showed that, the bacteriostatic and bactericidal concentrations for only thyme oil which showed the greatest zones of inhibition. The results revealed that E. coli no (3 u) showed the highest MIC and MBC, it was 250ug.

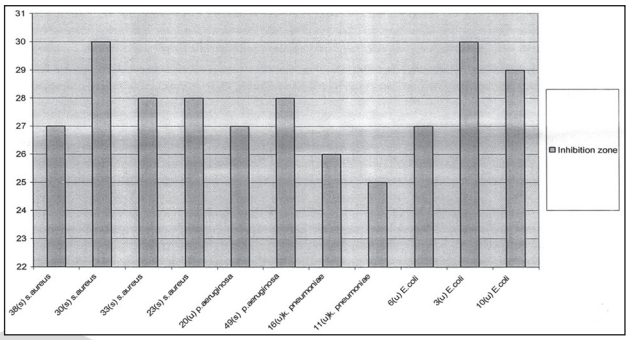

Figure 3. Efficiency of combination between thyme oil and imepenem antibiotic on the growth of selected bacteria.

Figure (3) classified the thyme oil when combined with the active antibiotic (Imipenem) for increasing the antimicrobial potentiality against tested isolates. It has been found the combination treatment was greater than that of each agent alone against all the selected isolates.

Discussion

The development of antibiotics for chemotherapy of bacterial infections represents one of the most remarkable achievements of this century. In the present study, a total of 50 bacterial isolates were collected from 50 patients in the cancer Department of King Abdul Aziz University Hospitals. The present study showed high level of resistance among S. aureus, E. coli, K. pneumoniae and P. aeruginosa isolates to cephalosprins, resistance to drugs was reported in several studies worldwide (22). The results showed that, the hightest percentage was obtained by S. aureus. Where was the prevalent isolated organism (36%).

The results were not in agreement with (23) they reported that low susceptibility rate (17% sensitivity) was found for Ampicillin. Also, these results were not matched with the study of Matsukawa et al. (24), who found that penicillins and first to second generation cephalosporin in vitro sensitivity tests must be undertaken as a guide for intelligent antimicrobial therapy. The carbapenem imipenem has proved to be an extremely useful antibacterial agent because of its great beta- lactamase stability and high intrinsic activity against' a broad range of bacteria (25, 26, 27, 28).

Multidrug resistance (MDR) is emerging problem in the clinical management of bacterial in-

fections. Enterobacteriaceae isolates resistant to multiple antibiotics have been reported from several parts in the world. (29).

However, the unstable nature of plasmids that can spread even to multiple species of bacteria and be lost or acquired spontaneously (30), made plasmid fingerprinting. Which was the first molecular typing method to be used for epidemiological purposes often poorly reproducible (31, 32).

P. aeruginosa is an opportunistic pathogenic bacteria which is usually very hard to control by antibiotic therapy. Resistance of P. aeruginosa to most antibiotics is a cardinal feature of this organism (33, 34). A major problem in the treatment of P. aeruginosa infections is the intrinsic resistance of these bacteria to a number of structurally unrelated antimicrobial agents (35, 36) Hence the knowledge of resistance pattern is essential.

The resistance of P. aeruginosa isolates to imipenem has been associated with increasing use of imipenem and occurrence of extended spectrum (3-lactamases in members of the family Enterobacteriacae. (25, 37, 38).

Our results detected that the susceptibility rates of Imipenem were 100 %, 93.3 %, 79.6% and 100 % for S. aureus, E. coli, K. pneumoniae, P. aeruginosa and. These results were in accordance with data reported in other studies of (39, 40, 41, 42).

Bonfiglio et al, (43) and Klein et al, (44) concluded that, the different levels of resistance found are in accordance with the low of high use of the drug in different centers. The appearance of new opportunistic microorganisms often multi resistant and the increasing resistance to antibiotics in well known pathogens (45, 46).

Resistance to antimicrobial agents may be mediated by genes that are encoded on the host cell chromosomes, plasmid and or into the chromosome (47, 48, 49).

These results were not matched with the study of Matsukawa et al (24), who found that, penicillins and first to second generation cephalosporin in vitro test must be taken as guide for intelligent antmicrobial therapy. The carbapenem imipenem has proved to be an extremely useful antibacterial agent because of it s great beta- lactamase stability and high intrinsic activity against broad range of bacteria (27). Multidrug resistance is emerging problem in clinical management of bacterial infections. Enterobacteriaceae isolates resistant to multiple antibiotics have been reported from several parts in the world (29).

P. aeruginosa is an opportunistic bacteria which is usually hard to control by antibiotic therapy. Resistance of P. aeruginosa to most antibiotics is a cardinal feature of this organism (12). A major problem in the treatment of P. aeruginosa infections is the intrinsic resistance of these bacteria to a number of structurally unrelated antimicrobial agents; hence the knowledge of resistance pattern is essential (50, 3).

Our study showed that S. aureus recorded high erythromycin resistance among MRSA isolates , it revealed that 27.7% of MARSA isolates were resistant to gentamycin. This does not agree with Tekwu et al (51) they reported an increase in gentamycin susceptible MARSA from 7.4 % to 64.8% and 94.4% in period between 1992 and 1998 in French hospitals, Felton et al, found that MARSA prevalence was approximately 36% .

In this study the isolated E. coli strain was tested diffusion method on Miller-Hinton medium , Production of (3-lactamase activity is well documented by several investigators (52,53).

Our results clearly showed that the essential oils of olive, castor, lavender and origanum exhibited slightly antimicrobial activity against the chosen multi resistant P.aeruginosa isolates. Al-Bayati (54) and Adam et al, (55) found that essential oils of origanum, lavandula and salvia showed antifungal properties against the human pathogen; Malassezia furfur, Trichophyton (older antibacterial agents) can be the essential oils of several plants as Thyme vulgaris was used as an antiseptic, other essential oils used as disinfectant action. For this purpose, they were extensively used by the ancient 1 Egyptians in the process of esmbalming with results which can be seen at the present day (56).

Deans and Richie (57) examined 50 volatile oils for then-antibacterial properties against genera of bacteria, using an agar diffusion technique. Sagdic (4) found that, two thyme (Thymus vulgaris, and Thymus serpyllum) had antibacterial activities and the most sensitive bacterial isolates against the spice hydrosols of thyme was S. aureus.

In the present study thyme, cinnamon, clove and caraway were most effective oils against more resistant MRSA isolates these results were agree-

ment with Zafra-Polo, et al. (58) who, reported that, the essential oil of Thymus leptophyllous showed higher antimicrobial activity against S. aureus and other tested bacteria as Staphylococcus aureus. The antimicrobial activity of different species of thymus was tested against bacteria and fungi the essential oil of Thymus broussonettii was most efficient for killing the microorganisms and inhibiting their growth (59). Also, Smith-palmer et al, (7) showed that Thyme. Cinnamon, Clove and bay oils were the most inhibitory against selected bacteria.

References

1. *Obeidat M, Shatnawi M, Al-Alawi M, et al. Antimicrobial Activity of Crude Extracts of Some Plant Leaves Research Journal of Microbiology, 2012; 7: 59 – 67.*
2. *Brook. Microbiology and management of infected solid tumours. Department of Pediatrics , George town School of Medicine Washington, DC, USA, Eur J. Cancer, 2007; 16(1): 12-6.*
3. *DeLeo RF, Chambers FH. Reemergence of antibiotic-resistant Staphylococcus aureus in the genomics era J Clin Invest. 2009; 119(9): 2464– 2474.*
4. *Sagdic O, Ozcan M. Antibacterial activity of Turkish spice hydrosols. Food. 2003; 14(3): 141-143.*
5. *Cirioni O, GMselli R, Silvestri C, et al. Tachyplesin 111, Colistin, and Imipenem against a multi resistant Pseudomonas Efficiency aeruginosa Strain. Antimicrob Agents Chemother. 2007; 51: 2005-2010*
6. *Smith- Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant and essences against five important food borne pathogens Letters in Applied Microbiology. 1998; 26(2): 118-122.*
7. *Hughes WT, Armstrong D, Bodey GP. Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clinical Infectious Disease. 2004; 34: 730-51*
8. *Tompkines LS. DNA methods in clinical microbiology in. E H Lennerte. A.Balows. V J Hauster. Jr and HShadomy (ed.). Manual: clinical B. microbiology.41thed. American Sc-cier.-For Microbiology'. Washington. D. C. 1985; 1023-1028.*
9. *Zinner SH. Antimicrobial Therapy in Cancer Patients: Studies from the EORTC International Antimicrobial Therapy Cooperative Group.Eur J. Of Cancer, 1997; Vol.33, Suppl.4: 544-549.*
10. *Lindsay JA, et al. Microarrays reveal that each of the ten dominant Lineages of Staphylococcus aureus has a unique combination of surface-associated And regulatory genes. J. Bacteriol. 2006; 188: 669-676.*
11. *Rahal JJ. Extended- spectrum p-lactamases:how big is the problem? Clin. Microbial. Infact. 2000; 6(suppl. 2):2-6.*
12. *Kumarasamya KK, Toleman AM, Walsh RT, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study, The Lancet Infectious Diseases, 2010; Volume 10, Issue 9: 597 – 602.*
13. *Schmitz FJ, Sadurski R, Kray A, et al. Prevalence of macrolide resistance genes in Staphylococcus aureus and Enterococcus faecium isolates from 24 European university hospitals. J. Antimicrob. Chemother, 2000; 45: 921-925.*
14. *Jacoby GA, Archer GL. New mechanisms of bacterial resistance to antimicrobial agents. N. Engl. J. Med. 1991; 324: 601-602*
15. *Nelson RR. In vitro activities of five plant essential oils against methicillin Resistant S.aweus and vancomycin resistant Enterococcus faecium. J. Antimicrob. Chemother, 1997; 40: 305-306.*
16. *Jarvis WR, Schlosser J, Chinn RY, et al. National prevalence of methicillin-resistant Staphylococcus aureus in inpatients US health care facilities, 2006. Am. J. Infect. Control. 2007; 35: 631-637.*
17. *Murray PR, Baron EJ, Pfaller MA, et al. Manual of clinical microbiology; American Society for Microbiology Edition, 1999; 14*
18. *Holt JG, Krieg NR, Sneath PH, et al. Bergy' Manual of Determination Bacteriology. 9thed. Williams and Willkins, Baltimore, Meryland. 1994.*
19. *Damato RF, Isenberg HD, Mckinley GA. Collaborative evaluation of the UniScept Qualitative antimicrobial susceptibility test .J.Clin.Micrbiolo. 1985.*
20. *Collee JG, Duguid JP, AG, Marmin BP. Mackie and MacCarthey .Practical Medical Microbiology 13th ed. Churchill Livingstone, Edinburgh, London, 1989; 2: 141-160*
21. *Ahmed FM. Antimicrobial studies of some essential oils on some Bacteria ; Thesis Master Degree , Faculty of Science ; Botany Department. 2004.*
22. *Klevens RM. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA. 2007; 298: 1763-1771.*

23. *Giacommetti A, Ciriani O, Schimizzi AM, et al. Epidemiology and microbiology of surgical wound infections.!. Clin. Microb. 2000; 38(2): 918-922.*

24. *Matsukawa M, Kunishinia Y, Takahashi S, et al. Staphylococcus uureus bacteriuria and surgical site infections by methicillin-resistant Staphylococcus aureus. Int. J. Antimicrob Agents, 2001; 17(4): 327-329.*

25. *Lipman B, Neu HC. Irmpenerr- an carbapenem antibiotic. Resistant . Med . Clin. North. Am. 1988; 72: 567- 579, Nature .227: 680 -685.*

26. *Wang C, Galandra GB, Aziz MA, Brown KR. Effecacy and safety of imipenern/Cilastatin: a review of world-wide clinical experience. Rev. Infect Dis. 1985; 7: 528-536.*

27. *Neu HC. Overview of mechanisms of bacterial resistance. BIAGN. Microbiol Inf. Dis, 1989; 12: 1095.*

28. *National Committee for Clinical Laboratorial Starndards-(NCCLS). Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Suplement, 2005; v.25, n.1, M100-S14.*

29. *Hall LM, Blok M, Bonders, et al. Multidrug resistance among Enterobacteriacae is Strongly Associated with the presence of integrons and is independent of species or isolate origin. J. Infect Dis. 2003; 187(2): 251-259.*

30. *Morlin GL, Hedges DL, Smith AL, Urns JL. Accuracy and cost of antibiotic Susceptibility testing of mixed morphology type of P. aeurginosa .J .Clin. Microbiol. 1994, 32: 1027-1030.*

31. *Stefani S, Agodi A, Russo G. Role of molecular methodologie in the epidemiologic studies infections. Ig. Mod. 1994; 102: 181-190.*

32. *Stefani S, Agodi A. Molecular epidemiology of antibiotic resistance. Int. I. Antimicrob. Agents. 2000; 13: 143-153.*

33. *Kadry AA. Antibiotic inactivating enzymes of certain strains of Pseiidomonas aeruginosa M. Sc, Thesis, Faculty of Pharmacy, Zagazig University, Egypt. 1985.*

34. *El-Dally SAE. A study on the proteolytic activity of pathogenic Psemiomonas acruginosa strains M. Sc Thesis. Faculty of Pharmacy. Zagazig University. Egypt. 1992.*

35. *Mouton J, Hollander J, Horrevorts A. Emergence of antibiotic resistance among Pseudomonas ncriiginosii isolates from patients with cystic fibrosis. J. Antimicrob. Chemother. 1993; 31:*

36. *Sparrow MTL, Warner M, Stefanidou M. Survey of resistance of Pseudomonas aeruginosa from UK patients with cystic fibrosis to six commonly prescribed antimicrobial agents. Thorax; 2003; 58: 794-796 .*

37. *Minami S, Akama M, Watanabe Y, et al. Imipenem and cephem resistant Pseudomonas aeruginosa carrying plasmids Coding for class B j3-lactamase . J . Antimicrobial . Chem. 1996; 37: 433-44*

38. *Spanu T, Luzzaro F, Perilli M, et al. Occurrence of extended spectrum p-lactamases in members of the family Enterobacteriacae in Italy: Implications for resistance to (3-lactams and other antimicrobial agents. Antimicrob. Agents Chemother. 2002; 46(1): 196-202.*

39. *Gowan JE, Jr Hall EC, Parrott PL. Antimicrobial susceptibility in Gram-negative bacteremia are nosocomial isolate more resistant.Antimicrobial Agents Chemother. 1989; 39(11): 645-649.*

40. *Amyes GB, Gemmell CG. Antibiotic resistance . J.Med. Microbiology. 1997; 46(2): 436-*

41. *Tohamy EV, Abo-Zeid AM, Shaheen AA, El-Awadi Satnah F. Nosocomial Infection in Surgical Hospital in Zagazig University. Arab Univ,Cairo. 2006; 14(1): 133-145.*

42. *Nickerson KE, West TE, Day PN, Peacock JS. Staphylococcus aureus Disease and drug resistance in resource-limited .The Lancet Infectious Diseases, 2009; 9(2): 130-135.*

43. *Bonfiglio G, Carciotto V, Rnsso G, Stefani S. Antibiotic resistance in P. aeruginosa, An Italian Survey. J. Antimicrob. Chemother. 1998; 41(5): 307-310.*

44. *Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin resistant Staphylococcus aureus, United States, 1999-2005. Emerg. Infect. Dis. 2007; 13: 1840-1846.*

45. *Stefani S, Agodi A. Molecular epidemiology of antibiotic resistance. Int. I. Antimicrob. Agents. 2000; 13: 143-153.*

46. *El Behedy EM, Shaheen AA, Amer FA, Fathy. Comparison of phenotypic and Genotype methods for detection of methicillin- resistant Staphylococcus aureus. countries in south and East Asia. M.D. Thesis, Microbiology and Immunology Department Faculty of Medicine Zagazig University, Egypt. 2000.*

47. *Agodi A, Jones C, Threlfall EJ.et al. Molecular characteristics of trimethoprim resistance in Shigella soneii in Sicily. Epidemiol. Infect. 1990; 105*

48. *Tenover F, McGowan J. Reasons for the emergence of antibiotic resistance. Am. J.Med. Sci. 1996; 311: 9-16. 29.*

49. *Mohammadreza VR. Chemical composition and antimicrobial activity of Pimpinella affinis. Ledeb. Essential oil growing in Iran. Int. J. Green Pharm. 2008; l 2 (3): 138-140.*

50. *Luzzaro F, Perilli M, Amicasante G, et al. Properties of multidrug resistant ,FSBL- producing Proteus mirabilis isolates and possible role of j3-lactamase inhibitor combinations. Int. J. Antimicrobial agents . 2001; 17(2): 131 Maraki et al, 2003.*

51. *Tekwu EM, Pieme AC, Beng VP. Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance. J Ethnopharmacol. 2012; 26; 142(1): 265-73. Epub 2012 May 11.*

52. *Hamouda HMH. Studies on antibiotic degradation by antibiotic-resistant bacteria. M. Sc. Thesis, Department of Botany, Faculty of science, Zagazig University, Egypt. 2000.*

53. *Aiello AE, Lowy FD, Wright LN, Larson EL. Meticillin-resistant Staphylococcus aureus among US prisoners and military personnel: review and recommendations for future studies. Lancet Infect. Dis. 2006; 6: 335-341.*

54. *Al-Bayati FA. Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts.J. Ethnopharmacol.March. 2008; 116(28): 403-406*

55. *Adam K, Sivropoulou A, Kokkini S, et al. 19 Antifungal activities of Origanum vulgare subsp. Hirtum menthe spicata, Lavandula angustifolia and Saviafroticosa essential oils against human pathogenic fungi J Agric Food Chem. 46: 1739-1745.*

56. *Alarm K, Maven H, Taie R, et al. Preliminary of sreaweeds, sea grass and lemongrass oils from papua New Guinea for antimicrobial and antifungal activities screening. International Journal of microbiology. 1994; 32(4): 396 -399*

57. *Deans SG, Richie G. Antibacterial properties of plant essential oils. Internatio. Journal of Food Microbiology. 1987; 5: 165- 180.*

58. *Zafra-Polo MC, Blazquez MA, Villar A. Spasmolytic and antimicrobial activity of the essential Oil from Thymus Leptophyllus and T. Webhianus. Fitoterapia, 1989; 60(5): 469-473.*

59. *Tantaoui EA, Lattaoui N, Errifi A, Benjilali B. Composition and antimicrobial activity of the essential oils of Thymus broitssonenii. T. Zygis and T.satureicides. Journal of Essential Oils Research. 1993; 5(1): 45-53.*

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Erythropoietin and transferrin metabolism in nephrotic syndrome patients

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Abstract

Objective: Renal excretion of erythropoietin and transferrin increases in nephrotic syndrome. We aimed to identify the relationship between anemia, and transferrin and erythropoietin metabolism.

Material and methods: This study included 30 nephrotic syndrome patients with normal glomerular filtration rates and creatinine levels; 15 of the patients were anemic. Serum erythropoietin, soluble transferrin receptor, 24-h protein excretion, albumin levels and urinary transferrin levels were analyzed in all the patients. Additionally, renal biopsy was performed in all the patients and the etiology of nephrotic syndrome was evaluated.

Results: There were statistically significant differences between the hemoglobin and hematocrit values; however, the difference was not significant in terms of mean corpuscular volume. Creatinine clearance, 24-h urinary protein and albumin, serum soluble transferrin receptor, and urinary transferrin values were similar between the groups; however, the erythropoietin level in the anemic group was higher. Moreover, urinary transferrin and 24-h urinary protein excretion were strongly correlated. Biopsy results were not correlated with anemia or erythropoietin.

Conclusion: As urinary transferrin and 24-h urinary protein are strongly correlated, proteinuria must be controlled for successful treatment of anemia. Erythropoietin and iron metabolism are greatly affected by glycoprotein metabolism; therefore, when proteinuria exists the treatment of anemia will be difficult.

Key words: Anemia, Erythropoietin, Iron metabolism, Nephrotic syndrome, Serum soluble transferrin receptor.

Introduction

Nephrotic syndrome (NS) is characterized by proteinuria, hypoalbuminemia, edema, hyperlipidemia, lipiduria, and hypercoagulability. In NS, along with other proteins, erythropoietin (EPO) and transferrin (TF) are also lost via urine, and plasma concentrations of proteins decrease[1, 2]. EPO is a glycoprotein weighting 30.4 kD. The normal plasma concentration is 10-25 U/l. Anemia, hypoxia, living at high altitudes, and a decrease in oxygenation of EPO-producing cells cause EPO to increase[3]. Hepatocytes, macrophages, and erythroblasts also secrete EPO[4].

TF is an 80-kD glycoprotein[5, 6]. Transfer of iron from absorption sites to storage is the main function of TF. TF is also a negative acute-phase protein[7, 8]. It is secreted primarily by the liver, as well as the testes, spleen, kidneys, and brain[9, 10]. The normal plasma concentration of TF is 2-4 g/l[10, 11]. Secretion of TF increases in response to uremia, hypoxia, iron deficiency, pregnancy, and estrogen[12]. Secretion of TF decreases in response to malnutrition, inflammation, and iron overdose[13]. In NS, TF decreases via urinary excretion and an increase in tubular catabolism[14]. This causes iron deficiency and microcytic anemia[15, 16]. TF is the primary carrier of iron to erythroid precursors. This means that in NS anemia can occur without iron deficiency. TF synthesis also increases in NS, but not enough to reach the normal level[14]. One reason for this is that TF is a negative acute-phase reactant, and in cases of malnutrition and chronic inflammation the level of TF decreases[17-19].

Circulating iron bound to TF is taken to into cells by TF receptors (TFRs), 80% of which are found in erythroid precursors and rapidly dividing cells such as those in the placenta[20]. Its plasma level increases when there is iron deficiency, erythropoiesis is induced by chemicals, or when

erythroblast volume increases[21-23]. The TFR that passes into the circulation is serum soluble TFR (SSTFR), which is a glycoprotein. The serum level is strongly correlated with TFRs and is affected by inflammation[24]. SSTFR is used to differentiate iron deficiency from anemia caused by chronic diseases. Plasma levels of SSTFR >1.5 g/l indicate iron deficiency with 70% specificity and 80% sensitivity[25]. The present study aimed to identify the relationship between anemia, and EPO and TF metabolism in NS.

Materials and Methods

Study design

The study included newly diagnosed 30 NS patients (16 male and 14 female). Hemoglobin levels <13 g/dl and 12 g/dl in males and females, respectively, were accepted as anemia. Renal and hepatic function tests were performed, and haptoglobin, serum iron, serum iron-binding capacity, and 24-h protein and albumin excretion were analyzed using an Olympus AU2700 biochemistry autoanalyzer (Olympus Life Science Europa, GmbH, Germany). Plasma levels of ferritin, vitamin B12, and folate were analyzed using an Abbott Architect i2000 hormone autoanalyzer (Abbott Laboratories Diagnostics Division, IL, USA) via chemiluminescence microparticle immune assay (CMIA). Sedimentation was analyzed using Vacuette-Greiner tubes and an SRS 100/2 Vacuette device. C-reactive protein was analyzed with a Dade Behring BNII device (Dade Behring Marburg, Germany), using the manufacturer's kits (Dade Behring [Cardiophase] CRP [hsCRP] kits) and the nephelometric method. Additionally, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin concentrations were analyzed. Urinary TF (UTF), SSTFR, and haptoglobin analyses were performed using a Dade Behring BN ProSpec device and the nephelometric method. Erythropoietin levels were analyzed manually using Biomerica (CA, USA) kits and Elisa. Creatinine clearance was calculated according to the Cockcroft-Gault formula. Following the provision of written informed consent, all the patients underwent renal biopsy, and the biopsy specimens were analyzed using immunofluorescence and light microscopy.

Statistical analysis

Data analysis was performed using SPSS v.13.0 for Windows. Continuous variables, standard deviations, and categorical variables are shown as percentages. Statistically significant differences between groups were analyzed using Student's t and Mann-Whitney U tests. The linear relationship between urinary albumin and protein level, and between serum erythropoietin and iron level were analyzed using Spearman's correlation test. To analyze the multivariate significance of the effect of serum iron and TF on anemia, logistic regression analysis was used. p values <0.05 were accepted as statistically significant.

Results

Among the 30 patients, 15 were anemic and 15 were not. Six patients were under angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment in both groups. Also there were six patients under immunosupressive or immunomodulator treatments in each groups. Evaluation of the renal biopsy specimens showed that 4 of the anemic patients had amyloidosis (AML), 5 had membranoproliferative glomerulonephritis (MPGN), 3 had mesangioproliferative glomerulonephritis (MSPGN), 2 had membranous glomerulonephritis (MGN), and 1 had focal segmental glomerulonephritis (FSGN), whereas 3 of the non-anemic patients had amyloidosis, 1 had membranoproliferative glomerulonephritis, 3 had mesangioproliferative glomerulonephritis, 4 had membranous glomerulonephritis, and 4 had focal segmental glomerulonephritis (Table 1).

Demographic features of patients and urea, creatinine, creatinine clearance, haptoglobin, albumin, serum lipids, sedimentation, CRP, and 24-h protein and albumin excretion levels, and liver function test results are shown in Table 2 and Table 3. There weren't any statistically significant differences between the anemic and non-anemic patients, except for alanine amino transferase (ALT) levels, which were statistically significantly lower in the anemic group ($p < 0.05$). Vitamin B12, folate, serum iron, serum iron binding capacity (SIBC), ferritin, SSTFR, EPO, and UTF levels in both groups are shown in Table 4. There was no statistically significant differences between the 2 groups.

Table 1. Distribution of the anemic and non-anemic patients according to biopsy results

	n	Anemic	(%)	Non-anemic	(%)	p
AML	7	4	57.1	3	42.9	
MPGN	6	5	83.3	1	16.7	
MSPGN	6	3	50.0	3	50.0	0.260
MGN	6	2	33.3	4	66.7	
FSGN	5	1	20.0	4	80.0	
Total	30	15		15		

AML: Amyloidosis; FSGN: focal segmental glomerulonephritis; MGN: membranous glomerulonephritis; MPGN: membranoproliferative glomerulonephritis; MSPGN: mesangial proliferative glomerulonephritis.

Table 2. Demographic features of patients

	Anemic	Non-anemic	Total
Age	32 ± 17	34 ± 18	34 ± 17
Gender	7 male, 8 female	9 male, 6 female	16 male, 14 female
ACEi or ARB treatment	6	6	12
IS or IM treatment	6	6	12

ACEİ: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; IS: immunosupressive; IM: immunomodulator.

Table 3. Biochemical test results in the anemic and non-anemic patients

Variables	Anemic	Non-anemic	p
Urea (10-50 mg dl^{-1})	42 ± 24	35 ± 19	0.436
Creatinine (0.6-1.3 mg dL^{-1})	1.04 ± 0.33	0.9 ± 0.24	0.233
Creatinine clearance (80-125 mL min^{-1})	100 ± 35	116 ± 34	0.217
Hemoglobin (14-17 mg dL^{-1}/12.5-15 mg dL^{-1} male/female)	10.1 ± 1.7	14.5 ± 1	<0.0001
Albumin (35-50 mg L^{-1})	33 ± 8.6	30 ± 9.7	0.875
LDL (0-100 mg dL^{-1})	123 ± 57	145 ± 59	0.572
HDL (35-85 mg dL^{-1})	40 ± 10	44 ± 16	0.091
Trigliserid (<150 mg dL^{-1})	122 ± 86	155 ± 97	0.265
Haptoglobin (30-200 mg dL^{-1})	279 ± 175	263 ± 99	1.000
Sedimentation (0-20 mm h^{-1})	61 ± 37	47 ± 26	0.285
CRP (0-5 mg L^{-1})	29 ± 48	8 ± 7	0.267
24-h urine protein (0-150 mg dL^{-1})	6658 ± 3446	6777 ± 3450	0.902
24-h urine albumin (0-30 mg dL^{-1})	2866 ± 1338	3042 ± 1626	0.870

Table 4. Hematological parameters in the anemic and non-anemic groups

	Anemic	Non-anemic	p
Vitamin B 12 (145-980 pg mL^{-1})	155 ± 59	210 ± 106	0.148
Folate (>2.7 ng mL^{-1})	3.8 ± 1.3	4.7 ± 1.5	0.161
Serum iron (25-155 µg dL^{-1})	36 ± 26	49 ± 22	0.126
SIBC (110-370 µg dL^{-1})	195 ± 98	240 ± 47	0.126
Ferritin (28-365 ng mL^{-1})	94 ± 106	89 ± 64	0.436
SSTFR (0.83-1.76 mg L^{-1})	2.3 ± 3.3	2 ± 2.6	0.514
EPO (4,5-33 mU mL^{-1})	13 ± 36	6 ± 4	0.079
UTF	14.5 ± 20	15 ± 17	0.506

EPO: Erythropoietin; SIBC: Serum iron binding capacity; SSTFR: Serum soluble transferrin receptor; UTF: Urinary transferrin.

In both the anemic and non-anemic groups Hb was not significantly correlated with EPO, UTF, or 24-h protein and albumin ($p>0.05$); however, in the anemic group, there was a significant, but inverse, correlation between Hb and sedimentation levels ($r=-608$, $p=0.016$). In the anemic group there was a direct and strong correlation between EPO and SSTFR levels ($r=792$, $p<0.0001$); however, this correlation was not observed in the non-anemic group. Additionally, UTF, and 24-h protein and albumin excretion were strongly correlated ($r=803$, $p<0.0001$; $r=827$, $p<0.0001$, respectively), but this correlation was not observed in the non-anemic group.

Discussion

Nephrotic syndrome is a disease in which albumin, TF, and many other proteins are excreted via urine[1]. In the present study we observed a strong correlation between UTF, and 24-h protein and albumin excretion. This result is consistent with most other studies[15, 16, 26-28]. This suggests that proteinuria might influence urinary excretion of TF, so at heavy proteinuria hypotransferrinemia occurs distinctively. Moreover, hypotransferrinemia causes anemia. Urinary excretion of TF increased in all the NS patients, but did not cause anemia. There wasn't a statistically significant difference between the anemic and non-anemic groups, in terms of UTF, or 24-h albumin and protein excretion levels. As such, we think that the occurrence of anemia in the NS patients was related to UTF loss, but that it was not the only factor.

When the underlying pathologies in the 2 groups were investigated, the incidence of anemia was higher among the patients with MPGN, but the difference was not statistically significant; as such, additional research is needed. Hypotransferrinemia leads to disturbance in the absorption, transport, and storage of iron—this causes anemia without iron deficiency Previous studies have shown that anemia did not correlate with iron depletion and that hypotransferrinemia may have been the cause[1]. In the present study the UTF value was 20% higher in the anemic group than in the non-anemic group; however, the difference was not statistically significant. This may have been due to the wide variation in UTF or because TF is a negative acute-phase reactant. The CRP and sedimentation levels in the anemic group were higher than those in the non-anemic group; therefore, we think that due to the decrease in TF in the anemic group UTF may not have been as high as expected.

Although serum TF levels were not measured, it would not be a surprise that transferrinuria as much as this cause hypotransferrinemia. Nonetheless, it is interesting that not all the transferrinuria patients were anemic. As such, one of the causes of anemia in the NS patients may have been hypotransferrinemia, while another factor may have been the primary disease itself.

One of the most important causes of anemia in NS patients is EPO deficiency[1]. In the present study a statistically significant difference in EPO levels between the anemic and non-anemic NS patients was not observed. The present study included patients with normal glomerular filtration levels so as to exclude EPO deficiency due to renal failure. Previous studies have noted that in NS the plasma level of EPO decreases and that this causes anemia[29]. Some animal studies reported that in rats with NS acute hypoxia led to a negligible increase in plasma EPO levels, while urinary excretion increased significantly, whereas in non-NS rats the plasma EPO level increased significantly while no evident increase in urinary excretion was observed[30]. The plasma EPO level in our anemic group was higher than that in the non-anemic group, but interestingly the mean EPO level in the anemic group was within the normal range and the mean EPO level in the non-anemic group was within the normal range. This is important because anemia did not occur despite low EPO and TF levels.

The observed increase in EPO in the anemic group was not as much as expected. One reason may have been the excretion of EPO due to proteinuria. Many studies have shown that anemia may improve by controlling proteinuria. Measurement of SSTFR showed that it was higher in the anemic group than in the non-anemic group; the difference was not statistically significant because of the wide variation, but it was notable. SSTFR levels in 3 of the 15 anemic patients were within the normal range, versus in 7 of the 15 patients in the non-anemic group. As such, SSTFR increased in 80% of the anemic group as a response to anemia, and in the anemic group there was a strong correlation between EPO and SSTFR ($p<0.0001$). In

the non-anemic group SSTFR increased in 53.3% of patients, possibly because of increased EPO activity or because higher SSTFR levels may have led to the maintenance of normal Hb levels. Moreover, these patients may have had a higher risk for anemia; therefore, SSTFR had 86% sensitivity and 66% specificity for anemia.

Conclusion

Severe proteinuria may occur in NS patients and albumin, TF, EPO, and other protein deficiency may be observed. The best way to treat anemia is to control proteinuria. This can be accomplished by controlling the primary disease—NS. When complex iron metabolism and proteinuria are considered together, we think that iron and EPO repletion alone may not be sufficient to treat anemia in NS patents.

References

1. *Vaziri ND. Erythropoietin and transferrin metabolism in nephrotic syndrome. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2001; 38: 1-8*
2. *Egrie JC BJ. The molecular biology of erythropoietin. In: Erslev AJ AJ, Eschbach JW, Winearls CG., ed. Erythropoietin-molecular, cellular, and clinical biology. Baltimore: Johns Hopkins University Press; 1991: 21-40.*
3. *Erslev AJ, Caro J, Miller O, Silver R. Plasma erythropoietin in health and disease. Annals of clinical and laboratory science. 1980; 10: 250-257*
4. *Erslev AJ, Caro J, Kansu E, Silver R. Renal and extrarenal erythropoietin production in anaemic rats. British journal of haematology. 1980; 45: 65-72*
5. *Lash A, Saleem A. Iron metabolism and its regulation. A review. Annals of clinical and laboratory science. 1995; 25: 20-30*
6. *Bonkovsky HL. Iron and the liver. The American journal of the medical sciences. 1991; 301: 32-43*
7. *Schreiber G, Dryburgh H, Millership A, et al. The synthesis and secretion of rat transferrin. The Journal of biological chemistry. 1979; 254: 12013-12019*
8. *Schaeffer E, Boissier F, Py MC, et al. Cell type-specific expression of the human transferrin gene. Role of promoter, negative, and enhancer elements. The Journal of biological chemistry. 1989; 264: 7153-7160*
9. *Idzerda RL, Huebers H, Finch CA, McKnight GS. Rat transferrin gene expression: Tissue-specific regulation by iron deficiency. Proceedings of the National Academy of Sciences of the United States of America. 1986; 83: 3723-3727*
10. *Bloch B, Popovici T, Chouham S, et al. Transferrin gene expression in choroid plexus of the adult rat brain. Brain research bulletin. 1987; 18: 573-576*
11. *Ritchie RF, Palomaki GE, Neveux LM, et al. Reference distributions for the negative acute-phase serum proteins, albumin, transferrin and transthyretin: A practical, simple and clinically relevant approach in a large cohort. Journal of clinical laboratory analysis. 1999; 13: 273-279*
12. *Burtis CA AE. Proteins. In: Burtis CA AE, ed. Tietz textbook of clinical chemistry. Philadelphia: WB Saunders; 1999: 477-540.*
13. *Ponka P, Beaumont C, Richardson DR. Function and regulation of transferrin and ferritin. Seminars in hematology. 1998; 35: 35-54*
14. *Jensen H, Bro-Jorgensen K, Jarnum S, et al. Transferrin metabolism in the nephrotic syndrome and in protein-losing gastroenteropathy. Scandinavian journal of clinical and laboratory investigation. 1968; 21: 293-304*
15. *Rifkind D, Kravetz HM, Knight V, Schade AL. Urinary excretion of iron-binding protein in the nephrotic syndrome. The New England journal of medicine. 1961; 265: 115-118*
16. *Hancock DE, Onstad JW, Wolf PL. Transferrin loss into the urine with hypochromic, microcytic anemia. American journal of clinical pathology. 1976; 65: 73-78*
17. *Kaysen GA, Sun X, Jones H, et al. Non-iron mediated alteration in hepatic transferrin gene expression in the nephrotic rat. Kidney international. 1995; 47: 1068-1077*
18. *Morlese JF, Forrester T, Del Rosario M, et al. Transferrin kinetics are altered in children with severe protein-energy malnutrition. The Journal of nutrition. 1997; 127: 1469-1474*
19. *Beaumier DL, Caldwell MA, Holbein BE. Inflammation triggers hypoferremia and de novo synthesis of serum transferrin and ceruloplasmin in mice. Infection and immunity. 1984; 46: 489-494*
20. *Beutler E, Hoffbrand AV, Cook JD. Iron deficiency and overload. Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program. 2003: 40-61*

21. *Wish JB. Assessing iron status: Beyond serum ferritin and transferrin saturation. Clinical journal of the American Society of Nephrology : CJASN. 2006; 1 Suppl 1: S4-8*

22. *Chiang WC, Tsai TJ, Chen YM, et al. Serum soluble transferrin receptor reflects erythropoiesis but not iron availability in erythropoietin-treated chronic hemodialysis patients. Clinical nephrology. 2002; 58: 363-369*

23. *Tarng DC, Huang TP. Determinants of circulating soluble transferrin receptor level in chronic haemodialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2002; 17: 1063-1069*

24. *Beerenhout C, Bekers O, Kooman JP, et al. A comparison between the soluble transferrin receptor, transferrin saturation and serum ferritin as markers of iron state in hemodialysis patients. Nephron. 2002; 92: 32-35*

25. *Tessitore N, Solero GP, Lippi G, et al. The role of iron status markers in predicting response to intravenous iron in haemodialysis patients on maintenance erythropoietin. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association. 2001; 16: 1416-1423*

26. *Howard RL, Buddington B, Alfrey AC. Urinary albumin, transferrin and iron excretion in diabetic patients. Kidney international. 1991; 40: 923-926*

27. *Kaysen GA. Plasma composition in the nephrotic syndrome. American journal of nephrology. 1993; 13: 347-359*

28. *Warshaw BL, Check IJ, Hymes LC, DiRusso SC. Decreased serum transferrin concentration in children with the nephrotic syndrome: Effect on lymphocyte proliferation and correlation with serum immunoglobulin levels. Clinical immunology and immunopathology. 1984; 33: 210-219*

29. *Vaziri ND, Kaupke CJ, Barton CH, Gonzales E. Plasma concentration and urinary excretion of erythropoietin in adult nephrotic syndrome. The American journal of medicine. 1992; 92: 35-40*

30. *Zhou XJ, Vaziri ND. Erythropoietin metabolism and pharmacokinetics in experimental nephrosis. The American journal of physiology. 1992; 263: F812-815*

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Vitreous humor between-eyes difference

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Abstract

Objective: The aim of this study is to analyze post-mortem concentration of potassium, sodium and chloride in vitreous of same pair of the eyes, aspirated and analyzed at the same time.

Material and Methods: This is retrospective study of biochemical analyses of vitreous samples of n=100 cases, collected at autopsies which were performed at Institute of forensic medicine, Medical faculty, University of Ljubljana. All samples from right and left eye are collected and analyzed at the same time. In all cases included in this study potassium, sodium and chloride concentrations were measured. All vitreous samples are collected respecting Coe's method. Approximately 2-3 ml of vitreous was aspirated in each case. Analyses were performed using Olympus AU 400 automatic analyzer, by Ion-selective methodology.

Results: The results of the present study suggest that concentration differences between eyes, at identical post-mortem interval, are not significant. The highest one sample test result is measured for potassium, which means potassium left – right concentration differences are close to zero, which is default value of the test. For chloride the result of one sample test is the smallest, however, p value of one sample test is higher than 0, 05 which means there are no statistically significant difference in their between eyes concentrations. All coefficients of correlation are high and significant, which confirms there are no statistically significant between eyes difference for concentration of potassium, sodium and chloride.

Conclusion: Present study resolves the issue of between eyes difference at identical post-mortem interval for vitreous electrolytes potassium, sodium and chloride. The results of this study clearly suggest these differences to be insignificant and therefore the validity of post-mortem vitreous humor analysis in forensic pathology applications cannot be questioned solely on the basis of these differences.

Key words: Vitreous humor, electrolytes, between the eyes difference, biochemical analyses.

Introduction

The autopsy is a medical procedure and golden standard for establishing cause of death, as well as quality of care assessment and improvement. In this modern era, many ancillary studies are routinely used during postmortem investigations. Over the years, the use and addition of ancillary laboratory studies has greatly enhanced the utility of the autopsy. An important and frequently utilized ancillary study is post-mortem vitreous biochemical analysis (1).

Vitreous humor, is viscous, colorless, clear gel, composed mainly of water (>90%), glucose, inorganic salts, hyaluronic acid, type II collagen fibers, and ascorbic acid. It is acellular and relatively isolated, rendering it is less susceptible than blood to biochemical changes, bacterial degradation, and contamination (2). Due to its post-mortem stability vitreous humor has high utility in forensic pathology (3-6)

When using older technological procedures a large number of investigators found no apparent discrepancies in chemical values between two eyes of individuals, when specimens from each eye where withdrawn simultaneously (7- 8).

With employment of newer methodologies vitreous analyses and validity of vitreous biochemistry in forensic applications has been questioned in the light of the reported concentration differences of various biochemical constituents, in the same pair of eyes, at identical post-mortem interval. In the study of Balasoorya et al, done in 60 individuals, whose vitreous specimens where withdrawn from both eyes, at the same time, that there where variations between the eyes in concentration of potassium, sodium and/or urates (9). The values of potassium, when measured by ion selective electrode, rather than flame photometry varied by

> 10% from the mean values. Madea et al., (1986), Sparks et al., (1989), also found statistically significant difference in between eye concentration (10, 11). The causes are not fully understood.

Mulla et al. (2005) analyzed potassium, sodium, chloride and calcium using a Beckman Coulter LX 20 Automated Analyzer, based on ion selective electrode methodology. They found no statistically significant between eyes differences at identical postmortem interval. A significantly high correlation was observed between paired potassium concentrations of both the eyes. A highly significant linear correlation was observed between the individual eye and mean potassium concentrations of both of the eyes with post-mortem interval. The observed differences were not significantly correlated with post-mortem interval. The results demonstrated that the between eyes differences for vitreous electrolytes and calcium are insignificant (12).

There still occur lacunae in the present knowledge as biochemical analyses of vitreous humor constituents by Ion selective electrode method has been used only by few authors. Not many studies have been conducted to compare Vitreous Biochemical changes between the two eyes (13).

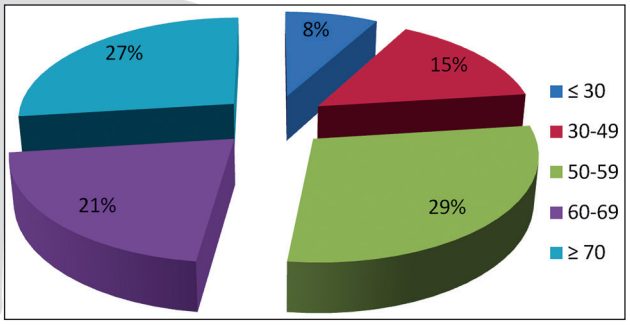
Present study was conducted to investigate the differences of electrolyte concentration (potassium, sodium and chloride) in vitreous in the same pair of eyes at identical post-mortem interval (PMI).

Materials and Methods

This is a retrospective study of biochemical analyses of vitreous samples from 100 individuals, collected at autopsies which were performed at the Institute of Forensic Medicine, Medical Faculty, University of Ljubljana. All samples from right and left eye are collected and analyzed at the same time. Cases with no available vitreous from both eyes were excluded. In all cases included in this study biochemical analyses of potassium, sodium and chloride concentrations are measured immediately upon sampling. When biochemical analyses had to be delayed, in a smaller number of cases, the samples were refrigerated at $+4^0$ C, up to 4 hours, without freezing. All vitreous samples are collected respecting Coe's method (4), separately, via scleral puncture, at the lateral canthus. All samples are aspirated slowly and gradually, in order to prevent disrupting of surrounding structures, which could lead to possible misinterpretation of measured concentration, for some of the vitreous constituents (4). Approximately 2-3 ml of vitreous was aspirated in each case. All analyses were performed using Olympus AU 400 automatic analyzer by Ion-selective methodology. All data are recorded in data base made in Microsoft Excel program 2010, separately for right and left eye in all cases. Statistical analyses were performed in SPSS 17.0 statistical program. All data were analyzed using descriptive statistic, Kolmogorov-Smirnov test for normality of distribution analyses, Wilcoxon test for variable which didn't satisfy normal distribution and t-test for variables which satisfies normal distribution. One sample test was used for testing differences between left and right eye electrolytes concentrations compared with default value, which is in this case zero, Spearman's rank correlation coefficient for testing association between left and right eye potassium concentration differences and Pearson linear correlation coefficient was used for testing association between left and right eye sodium and chloride concentration difference.

Results

Graph 1. represents sample structure according to the age. 77% of the sample was presented with samples from persons olden than 50. Average age of the sample is 59,56 with SD = ± 14,82 years[1].



Graph 1. Age distribution of the sample

Concentrations of potassium (K^+), sodium (Na^+) and chloride (Cl^-) were analyzed in vitreous of both eyes in all 100 cases included in this study. According to the Kolmogorov-Smirnov test

1 For the determination of average values we excluded 1 case – 9 month old child.

(K-S), which tested normal distribution, measured concentrations of potassium do not satisfied normal distribution ($p < 0,05$), and therefore Wilcoxon test was used to test the differences. Chloride and Sodium ($p > 0,05$) satisfies normal distribution and in those cases t-test was used (Table 1). Wilcoxon test, showed no statistically significant differences between the eyes potassium concentration ($p > 0,05$). According to the results of t-test, there is no statistically significant between the eyes chloride, or between the eyes sodium concentration difference. The results of one sample test differences between left and right eye electrolytes concentrations, presented in Table 2 do not significantly differ from zero, which is default value for the test. The results allow conclusion that

Table 1. Descriptive statistic results for Potassium, Chloride and Sodium concentrations of the sample and KS test

	Min.	Max.	Mean	Std. Deviation	Kolmogorov-Smirnov Z	p value - Asymp. Sig. (2-tailed)
Potassium D	5,000	44,000	12,080	6,365	1,725	0,005
Potassium L	4,000	44,000	12,070	6,580	1,746	0,005
Potassium M	5,000	44,000	12,075	6,431	1,564	0,015
Chloride R	75,000	145,000	109,060	13,580	0,897	0,397
Chloride L	64,000	188,000	108,540	15,646	1,255	0,086
Chloride M	79,000	166,500	108,800	13,564	1,082	0,192
Sodium R	92,000	170,000	130,890	14,352	1,032	0,237
Sodium L	77,000	218,000	130,720	17,685	1,248	0,089
Sodium M	92,000	192,000	130,805	15,234	1,114	0,167
Potassium L-R	-5,000	6,000	-0,010	1,480	2,527	0,000
Chloride L-R	-43,000	65,000	-0,520	11,070	2,566	0,000
Sodium L-R	-52,000	37,000	-0,170	10,447	2,077	0,000

KS - Kolmogorov-Smirnov test, R – Right side and L – left side measurements, M - average value of the measurements and L-R for left – right differences. All concentrations are expressed in mmol/L., Sig. – significant

Table 2. One-Sample Test

	Test Value = 0					
	Descriptive statistics				95% Confidence Interval of the Difference	
	t	df*	Sig. (2-tailed)	Mean Difference	Lower	Upper
Potassium (L-R)	-0,068	99	**0,946**	-0,010	-0,3038	0,2838
Chloride (L-R)	-0,470	99	**0,640**	-0,520	-2,7164	1,6764
Sodium (L-R)	-0,163	99	**0,871**	-0,170	-2,2428	1,9028

**df – degree of the freedom*

Table 3. Correlation for potassium

			Potassium R	Potassium L	Potassium S
Spearman's rho	Potassium R	Correlation Coefficient	1,000	,948**	,984**
		Sig. (2-tailed)	.	,000	,000
		N	100	100	100
	Potassium L	Correlation Coefficient	,948**	1,000	,989**
		Sig. (2-tailed)	,000	.	,000
		N	100	100	100
	Potassium M	Correlation Coefficient	,984**	,989**	1,000
		Sig. (2-tailed)	,000	,000	.
		N	100	100	100

***. Correlation is significant at the 0.01 level (2-tailed) and 0.05 level (2-tailed).*
Rho – coefficient of correlation of rang

there are no statistically significant difference in potassium, chloride and sodium between the eyes concentrations. Variable which didn't satisfy normal distribution (potassium concentrations) were tested with correlation of rang test (Table 3) and variables with normal distribution (sodium and chloride) were tested with coefficient of linear correlation (Table 4 and Table 5). All coefficients of correlations are high and statistically significant which confirms there are no statistically significant between the eyes concentration differences of potassium, sodium and chloride.

Discussion

The results of the present study show that between–eye concentration differences, at identical post-mortem interval are not significant. Results of One sample test shows potassium left-right vitreous concentration difference (-0,068) is the nearest to the default value of the test, which is zero. For sodium left-right concentration difference from zero is -0,163 and the biggest left-right concentration difference from zero is obtained for chloride (-0,470). It was concluded that the obtained left-right concentration difference from zero are not statistically significant, in other words, we can say that those difference do not exists.

Some early studies suggested near identical biochemical values for the two eyes (4, 15). These authors, however, didn't provide any statistical explanation. In this study it is observed identical values in only a small number of the paired samples analyzed.

All results in this study are tested with appropriate coefficient of correlation tests, Spearman correlation test for sodium and chloride and Pearson correlation test for potassium. All coefficients of correlation are high and significant. The results of correlation tests confirm there are no statistically significant differences in between the eyes electrolytes concentration.

Table 4. Correlation of the measurement of chloride

		Chloride R	Chloride L	Chloride S
Chloride R	Pearson Correlation	1	,722**	,917**
	Sig. (2-tailed)		,000	,000
	N	100	100	100
Chloride L	Pearson Correlation	,722**	1	,938**
	Sig. (2-tailed)	,000		,000
	N	100	100	100
Chloride M	Pearson Correlation	,917**	,938**	1
	Sig. (2-tailed)	,000	,000	
	N	100	100	100

***. Correlation is significant at the 0.01 level (2-tailed) and 0.05 level (2-tailed).*
R = right, L= left, M= average value, sig.- significant

Table 5. Correlation of the measurement for sodium

		sodium R	sodium L	sodium M
Sodium R	Pearson Correlation	1	,807**	,939**
	Sig. (2-tailed)		,000	,000
	N	100	100	100
Sodium L	Pearson Correlation	,807**	1	,961**
	Sig. (2-tailed)	,000		,000
	N	100	100	100
Sodium M	Pearson Correlation	,939**	,961**	1
	Sig. (2-tailed)	,000	,000	
	N	100	100	100

***. Correlation is significant at the 0.01 level (2-tailed) and 0.05 level (2-tailed).*
R = right, L= left, M= average value, sig. significant

The present study findings are consistent with the conclusion of Tagliero et al., (2001) and Munos et al., (2005). In Tagliero et al., (2001), study authors confirmed, through a microsampling technique, and capillary electrophoresis, that no statistically significant differences exist for potassium in two eyes of a person (17). Munos et al., (2005), confirmed no statistically significant differences for electrolytes Na, K, Cl and for Calcium. All biochemical analyses in their study were done by ion selective electrode methodology (12).

Microsampling technique used which employed aspiration of microliter amounts of vitreous in Tagliero et al. study (17) and complete vitreous aspiration technique used by Mula et al., are different from the 2-3 ml of vitreous humor aspirated for biochemical analyzes used in this study. This technique avoids possibility of tearing fine tissue structure that surround vitreous, which could influence the measurements (18).

The results of this study could not confirm some previous studies, which suggested high between the eyes differences for vitreous constituents including potassium (6, 9, and 16). Pounder et al., (1998), reported significant between the eyes potassium concentration differences for potassium (16). Present study does not support this conclusion about vitreous potassium, but are in agreement with the findings of no significant differences in the same pair of eyes for sodium and chloride.

A principal reason for conflicting reports about between the eyes differences at identical postmortem interval may be the variations in study methods and possible sample manipulations before analyses. An obvious discrepancy may be the aspiration technique adopted by some investigators. It has been suggested that the concentration of vitreous solutes next to retina is different than the concentration in the central portion of the globe, and therefore, it is essential to aspirate vitreous humor, as completely as possible, to reflect accurately the concentration levels of all solutes (14) The aspiration technique employed by Balasooriya et al. (1984) as they aspirated only the initial 1 ml of vitreous could distort concentration levels of the solutes (9). Pounder at al. (1998) assessed the effect of the sampling technique on the observed between the eyes difference by aspirating vitreous humor in two installments and did not find any major influence of the sampling technique (16). Madea et al. (1989) strictly followed Coe's recommendations (4) and still observed high between-eyes differences (10).

The results of Thierauf et al study (2009) prove slight differences in the analytical results caused by variations in the sample pre-treatment and show options to improve the precision (19).

It has been already reported by Coe and Apple (1985) that a variation in values occurs as a result of the instrumentation (5). The biochemical analyses used in this study was done on automatic analyzer, by Ion selective electrode method, which is similar to the biochemical analyses used by Pounder et al. (16) and by Mula et al. (12)

Differences in concentration of various parameters reported in some studies may be explained by the time lag between vitreous sample collection and the analyses. In some studies samples are kept frozen at 70^0 C, before biochemical analyzes (10). The inconsistent storage conditions may have influenced the results to a certain degree and it is suspected that after indefinite storage at low temperatures, results may not accurately represent the biochemical concentrations of the vitreous humor (12). In the present study, suspected influence of sample storage on vitreous humor biochemical values was eliminated by immediate biochemical analyses of the samples in most of the cases. When biochemical analyses had to be delayed, in a smaller number of cases, samples were stored at + 4^0 C, up to 4 hours, without freezing. The present study technique of immediate post-collection analyses is in accordance with the technique adopted by Pounder et al. (16) and by Mula et al. (12). However, in spite of a similar technique Pounder et al. (16) reported significant and erratic between the eyes difference for potassium. In the past, for the calculation of the post-mortem interval potassium has mainly been used and this finding raised question on validity of vitreous biochemistry, particularly the use of potassium concentration in postmortem interval estimation. The present study resolves that issue and confirms that vitreous biochemistry cannot be questioned solely on the basis of potassium or other electrolyte between the eyes concentration differences.

Conclusion

Post-mortem biochemistry is becoming increasingly essential in the forensic pathology routine and considerable progress has been made over the past years (20). Present study resolves the issue of between the eyes difference at identical post-mortem interval for vitreous electrolytes: potassium, sodium and chloride. This study clearly confirms differences between the eyes concentration of potassium, sodium and chloride to be insignificant and therefore the validity of post-mortem vitreous humor analysis in forensic pathology applications cannot be questioned on the basis of these differences.

Further systemic investigations are necessary to evaluate the influence of the pre-analytical treatment and the measurement apparatus and – as the case may be – to estimate the post-mortem interval in careful consideration of the manifold influencing variables (19).

References

1. *Rose KL, Collins KA. Vitreous chemical analysis. Newes Path 2011. Available from URL: http: //emedicine.medscape.com/article/1966150-overview*
2. *Stephens RJ, Richards RG. Vitreous humor chemistry: the use of potassium concentration for the prediction of the postmortem interval. J Forensic Sci 1987; 32: 503-9.*
3. *Jaffe FA. Chemical Postmortem changes in the intraocular fluid. J Forensic Sci 1962; 7: 231-237.*
4. *Coe JI. Postmortem chemistries on human vitreous humor. Am J Clin Pathol 1969; 51: 741-50.*
5. *Coe JI, Apple FS. Variations in vitreous humor chemical values as a result of instrumentation. J Forensic Sci 1985; 30: 828-35.*
6. *Madea B, Musshoff F. Posmortem biochemistry. Foresic Sci Int 2007; 165: 165-177.*
7. *Adelson L, Sunshine I, Rushforth NB, Mankoff M. Vitreous potassium concentration as an indicator of the postmortem interval. J Forensic Sci 1963; 503-14.*
8. *Forman DT. Electrolytes of the vitreous humor as a measure of the postmortem Interval. Clin. Chem. 1980; 1042-6.*
9. *Balasooriya BAW, Hill CA, Williams AR. The biochemistry of vitreous humour. A comparative study of potassium, sodium and urate concentration in eyes at identical time intervals after death. Forensic Sci Int 1984; 26-2: 85-91.*
10. *Madea B, Henssge C, Staak M. Postmortem increase in potassium in the vitreous humor. Which parameters are suitable as indicators of antemortem agonal electrolyte imbalance? Z Rechtsmed 1986; 97(4): 259-68.*
11. *Sparks DL, Oeltgen PR, Kryscio RJ, Hunsaker JC 3rd. Comparison of chemical methods for determining postmortem interval. J Forensic Sci 1989; 34: 197-206.*
12. *Mulla A, Massey KL, Kalra J. Vitreous humor biochemical constituents: evaluation of between the eyes differences. Am J Forensic Med Pathol 2005; 26: 146-9.*
13. *Sachdeva N, Rani Y, Singh R, Murari A. Estimation of Post-Mortem Interval from the changes in Vitreous bichemistry. J Indian Acad Forensic Med 2011; Vol 33 No 2.*
14. *Coe JI. Vitreous potassium as a measure of the postmortem interval: an historical review and critical evaluation. Forensic Sci Int 1989; 42: 201-13.*
15. *Sturner WQ, Gantner GE Jr. The postmortem interval. A study of potassium in the vitreous humor. Am J Clin Pathol 1964a; 42: 137-44.*
16. *Pounder DJ, Carson DO, Johnston K, Orihara Y. Electrolyte concentration differences between left and right vitreous humor samples. J Forensic Sci 1998; 43: 604-7.*
17. *Tagliaro F, Bortolotti F, Manetto G, Cittadini F, Pascali VL, Marigo M. Potassium concentration differences in the vitreous humour from the two eyes revisited by microanalysis with capillary electrophoresis. J Chromatogr A 2001; 924: 493-8*
18. *Bito LZ. Intraocular fluid dynamics. I. Steady-state concentration gradients of magnesium, potassium and calcium in relation to the sites and mechanisms of ocular cation transport processes, Exp Eye Res 1970; 10: 102–116.*
19. *Thierauf A, Musshoff F, Madea B. Post-mortem biochemical investigations of vitreous humor. Forensic Sci Int 2009; 78–82.*
20. *Palmiere C, Mangin P. Postmortem chemistry update part I. Int J Legal Med 2012; 126: 187-198.*

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Complementary and alternative medicine use among patients attending a dermatology clinic in Turkey

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Abstract

Context: Complementary and alternative medicine use is common among dermatology patients and most of them do not disclose their CAM use to their doctors.

Aims: The aim of the study was to examine the use of Complementary and Alternative Medicine among patients attending a hospital dermatology clinic in Turkey, and their satisfaction with Complementary and Alternative Medicine (CAM).

Methods and Material: A cross-sectional study was carried out between June-August 2011 among patients attending to out-patient clinic of İstanbul Medeniyet University Göztepe Training and Research Hospital Department of Dermatology. Data were collected by self administered questionnaires.

Statistical analysis used: Descriptive analyses and relevant significance tests were used for comparisons.

Results: Total 195 patients were included in the study. Of these participants, 134 were female, and 61 were male.Fifty-three patients (27.1%) had been used one of CAM methods in the previous year. Patients who were higher educated, had longer disease duration or were suffering acne vulgaris or psoriasis were more likley to have used CAM ($p<0.05$). Most commonly used CAM method was herbal remedies (60.4%). One-fourth (24.5%) of the participants stated that they had got any benefit. Nine patients (16.9%) declared that thay had some side effects.

Conclusions: CAM use is a common practice among dermatology patients in Turkey. Patients who are higher educated, have longer disease duration or are suffering acne vulgaris or psoriasis are more likely to use CAM. Taking into consideration of high prevalence of CAM use; dermatologists and primary care doctors should inquire about CAM use of their dermatology patients for preventing possible side effects and drug inreactions.

Key Words: Complementary and alternative medicine, dermatology, CAM use

Key Messages: CAM use is a common practice among dermatology patients in Turkey. Taking into consideration of high prevalence of CAM use; dermatologists and primary care doctors should inquire about CAM use of their dermatology patients for preventing possible side effects and drug inreactions,

Introduction

The National Center for Complementary and Alternative Medicine (NCCAM) defines Complementary and Alternative Medicine (CAM) as "medical and health systems, applications and products currently not considered as part of conventional medicine".[1]

An increased interest in CAM is observed among both the general population and health professionals.[2,3]A review indicated that the prevalence of CAM use in the general population ranged from 9 to 65 % worldwide.[4,5]

Skin diseases among adult population is one of the common reasons of CAM use.[6-9] According to survey data, 35 - 69% of patients with skin diseases have used complementary and alternative medicine (CAM) in their lifetime. In a very recent ovewiev, the authors have concluded that more evidence and better studies are needed for each of the major CAM modalities before they may be

considered as independent therapeutic options and dermatologists should obtain a thorough history of CAM use from their patients.[10]

There are few studies about CAM use among adult patients with dermatologic diseases.in Turkey. The aim of this study was to examine the use of CAM among patients attending a hospital dermatology outpatient clinic in Turkey, and their satisfaction with CAM use.

Subject and methods

This cross-sectional study was carried out between June - August 2011 among patients attending to out patient clinic of İstanbul Medeniyet University Göztepe Training and Research Hospital Department of Dermatology. Data were collected by using self-administered questionnaires. The self-administered questionnaire used was prepared by the researchers after review of the relevant literature, and finalized following a pilot study.

The questionnaire consisted of three parts. The first part included questions on sociodemographic characteristics of the patients, such as age and gender. The second part included questions about lifestyle and habits, self health perception, dermatologic diagnosis and duration of the disease. The third part included questions about experiences of CAM use and overall opinion rating scale about CAM use. A cover sheet that included definition and a list of CAM methods used in Turkey has been provided for every participant in the study to minimize recall bias and to avoid misunderstandings due to different definitions of and perceptions about CAM.

Total 220 patients over 18 yrs old and admitted to the outpatient clinic with any dermatologic complaint included in the study. Every patient was informed about the aim of the study and consents were taken. Twenty-five patients were excluded from the study because either refused to or have not completed the questionnaire. The research was approved by the Ethics Committee of Yeditepe University.

Data analysis

Statistical analyses were performed using SPSS v.19. Descriptive analyses and relevant significance tests were used for comparisons. Two-independent sample t test was used for comparison of means and chi-square test was used for comparison of distributions of categoric data. The level of significance was set at $p < 0.05$.

Results

Total 195 patients were included in the study. Of these participants, 134 were female, and 61 were male, and the mean age was 40.5 ± 16.3 years. Sociodemographic characteristics and health status of the patients included in the study are presented in Table 1. Table 2 presents the distribution of most frequent dermatologic diagnoses in the group.

Fifty-three patients (27.2%) had been used one of CAM methods in the previous year. The use of CAM methods found to be significantly different by the education level and the duration of the disease of the patients ($p=0.037$ and $p=0.010$ respectively) University graduate patients used CAM significantly more than the others and patients with disease duration 10-14 yrs or more than 15 yrs have used CAM more than the patients with shorter disease duration ($p<0,05$). [table 1]

Proportion of CAM users were significantly higher among the patients with acne vulgaris (50.0%, $p<0.05$) and psoriasis (44.5%, $p<0,05$) when compared with the others.

CAM methods that have been used by the patients are presented in Table2. [table 2]

Most frequently used methods are Herbal remedies (60.4 %). [table 3]

Table 4 presents the distribution of answers to the question related with CAM use. Most of the patients have used CAM after they had modern medicine treatment for sometime and most common purpose of use was to support the main treatment. Usually they applied the CAM methods by themselves. Major information sources of CAM users expressed to be friends and family members/ relatives. Most of the patients stated that they did not get any benefit or get moderate benefit of CAM use. [table 4]

Nine patients of total 53 (16.9%) declared that thay had some side effects due to CAM use,

20 patients (37.7%) disclosed about their CAM use to their doctors and 22 (41.5%) did not, others did not answered the question.

The reasons of not to disclose about their CAM use to their doctors were: " Because my doctor has not asked about it" (n= 18, 34%) ; "I have thought

Table 1. Sociodemographic characteristics and health status of the patients included in the study regarding CAM use

Characteristics	CAM users	Non CAM users	P value
Age	41.05±16.33	40.51±16.39	0.837
	n (%*)	***n (%*)***	
Gender Women Men	35 (26.1) 18 (29.5)	99 (73.9) 43 (70.5)	0.622
Marital Status Married Single	26 (22.4) 27 (35.1)	90 (77.6) 50 (64.9)	0.054
Education Illeterate Primary School High School University	4 (26.7) 16 (20.0) 12 (23,5) 21 (42.9)	11 (73.3) 64 (80.0) 39 (76.5) 28 (57.1)	**0.037**
Smoking Smoking Ex smoker No smoking	13 (27.7) 13 (35.1) 27 (24.3)	34 (72.3) 24 (64.9) 84 (75.7)	0.439
General Health Perception Very bad Bad Moderate Good Very good	2 (66.7) 6 (37.5) 20 (26.3) 24 (27.0) 1 (10.0)	1 (33.3) 10 (62.5) 56 (73.7) 65 (73.0) 9 (90.0)	0.316
Duration of the disease (yrs) <1 1 - 4 5 - 9 10 -14 > 15	5 (15.2) 13 (20.6) 8 (21.6) 10 (38.5) 17 (47.2)	28 (84.8) 50 (79.4) 29 (78.4) 16 (61.5) 19 (52.8)	**0.010**

** : Row percentage*

Table 2. Distribution of most frequent diagnoses by CAM use

Diagnosis	CAM users n (%*)	Non CAM users n (%*)	TOTAL n	P value*
Psoriasis	20 (44.5)	24 (54.5)	44	0,002
Acne vulgaris	8 (50.0)	8 (50.0)	16	0,032
Vitiligo	3 (30.0)	7 (70.0)	10	1,000
Chronic dermatitis	1 (12.5)	7 (87.5)	8	0,686
Atopic dermatitis	0 (0)	5 (100.0)	5	0,326
Others	21 (18.7)	91 (81.3)	112	0,014
Total	53 (27.2)	142 (52.8)	195	

** : Row percentages*

Table 3. Distribution of CAM methods among CAM users

CAM Methods	(n)	(%)
Herbal remedies	32	60.4
Balikli Göl*	6	11.3
Clay, mud, sulphur	6	11.3
Bioenergy	1	1.9
Praying	1	1.9
No answer	7	13.2
TOTAL	53	100

**It is name of a lake, famous with the curative effects of fish living in for psoriasis*

Table 4. Answers to the questions related with CAM use among CAM users

Questions - Answers	**Number (n)**	**Percentage (%)**
When did you use CAM method after you have got the diagnosis?		
- After I have learned my disease	7	13.2
- Prior to my treatment	7	13.2
- As supplementary following the start of my treatment	9	17.0
- Some time after the start of my treatment	24	45.3
- After I have tried all modern medical treatments	6	11.3
TOTAL	*53*	*100*
What was your purpose to use a CAM method?		
- As a supplementary to my treatment	30	56.6
- I did not believe modern medicine is sufficient	10	18.8
- - My doctor has suggested	3	5.7
- There are so many side effects of modern medicine	2	3.8
- There is no cure chance of my disease	3	5.7
- I am personally interested in CAM	5	9.4
TOTAL	*53*	*100*
Who applied the CAM method that you have used?		
- A medical doctor specialized on CAM	7	13.2
- Myself	26	49.0
- My primary health care physician	3	5.7
- A certified non medical person working on CAM	10	18.8
- A friend, a relative, a neighbourhood	4	7.6
- Other	3	5.7
TOTAL	*53*	*100*
Where did you hear the CAM method that you have used?		
- From a medical doctor	9	17.0
- From friends	17	32.1
- From family members and relatives	10	18.8
- Television, newspaper, internet	8	15.1
- Advertisement	2	3.8
- I myself explored	5	9.4
- Other	2	3.8
TOTAL	*53*	*100*
Do you think that you have got benefit from the CAM method you have used?		
- Not at all	13	24.5
- Some benefit	4	7.6
- Moderate benefit	18	33.9
- A lot benefit	2	3.8
- I have completely cured	3	5.7
- No reply/don't know	13	24.5
TOTAL	*53*	*100*

that my doctor would give negative reaction" (n= 6, 11.3%); " It could effect my medical treatment negatively" (n=6, 11.3%); " I have thought that my doctor could stop my treatment" (n=6, 11.3%). Others did not answered to the question.

19 patients (35.8%) stated that their doctors asked them about their CAM use and 30 patients (56.6%) stated that their doctors did not, 4 patients (7.5%) did not anwered the question. General opinion of the users about CAM was rated 4.5 ±3.1 on 10 points rating scale where "0" was completely negative and "10" was completely positive.

Discussion

CAM applications make up a significant portion of health spending and the demand for CAM applications is on the increase. Medical doctors, even if they are not CAM practitioners themselves and have negative attitudes towards CAM, will from time to time feel the need to be able to provide guidance for their patients and to know about CAM methods, at least at the minimal level of drug interactions and treatment effects. [11]

Dermatologic problems are the common reasons for CAM use. Prevalence of CAM use differs between 25-80 % among dermatology patients in different studies from different countries [6-9,12,13]; but in any case percentage of CAM use among dermatology patients is challenging enough to deserve special attention and to necessitate increased awareness among medical doctors.

Most of the previous studies conducted in Turkey focused on specific dermatologic conditions, so it is difficult to compare general CAM use among dermatology patients. In one of the study conducted with 1006 patients that had been attending to a dermatology clinic in a tertiary hospital, Gonul et al found that 33.5% of the patients had been used at least one method of CAM and the most common diagnoses were acne vulgaris, psoriasis, contact dermatitis and fungal infections among CAM users. [14] The most common methods that had been used were humectants, cologne, spiritual healing and herbal remedies. In another study conducted by Kutlu et al with 1000 patients attending to a dermatology clinic, it was found that 12.6% of patients had been used CAM for their dermatologic problem and most common diseases that patients used CAM for were psoriasis, acne vulgaris, alopecia, verruca. [15] The most common methods used were topical herbal remedies, praying and balneotherapy. In this study, 27.1 % of the patients had been used one of CAM methods for their dermatologic problems and patients with diagnoses of acne and psoriasis had used CAM more than the patients with other diagnoses. The most common method that had been used was herbal remedies which constituted 60% of all CAM use. These differences in usage rates may have been related to methodological differences and also to definition of CAM. As a result, it seems that CAM use is not an uncommon practice among dermatology patients in Turkey.

Some specific dermatologic diseases such as acne vulgaris, psoriasis, eczema and dermatitis have been related with CAM use more than others.[7,9,13,14] Also prevalence of CAM use might have increased when studied for a specific disease. Studies included patients with psoriasis showed life time CAM use ranging from 43 to 60%.[16,17]. In a study surveying 375 patients with acne vulgaris in Turkey, Bilgic and Ak have found 55.6% CAM use among patients which is a number quite higher than we have found in our study and other studies that have been included patients with any dermatologic problem.[18]

Along with specific diagnosis, duration of the disease also is an important factor to CAM use. Longer disease duration has been related increased prevalence of CAM use.[9,13,15]

Herbal remedies are foremost used CAM modality in literature as it is the case in this study.[12] In Turkey, praying also is a very common modality used to be cured in different disease entities making an important difference when compared to European countries and is especially used for verruca among dermatology patients. The use rate of praying as a CAM method was relatively low in the study group when compared to other studies. This may have been related to low numbers of patients in specific disease groups. Also, we have asked the last method that have been used in the previous year, not all the methods, although CAM users could have been used more than one CAM method during their disease process. Balikli gol is also another method specific to Turkey and commonly used by patients with psoriasis. There are differences among countries regarding

methods of CAM have been used commonly. For example Chinese medicine is not well known in Turkey and there are few practitioners of many CAM methods that have been defined by NCCAM. Studies surveying dermatology patients have shown that some methods have not been mentioned at all and in another national study surveying medical students some methods have not known by any student.[16,19]

Gender, marital status and education have been known to effect attitudes toward CAM use in general. Previous studies surveying general CAM use for different purposes, have found that women have more positive attitudes towards CAM, and are more likely to use it.[6] We did not find any gender difference regarding CAM use as it is the case in other two studies surveying dermatolgy patients in Turkey. In the study by Kutlu et al, they have found that young, single patients and patients with high education level preferred CAM more than others did; but Gonul et al have not found any relation between gender, age, education level and CAM use among dermatology patients.[15,16] Chen et al in their study found that patients 30-50 yrs old were less likely to report CAM use then younger and older age groups contrary to the findings of the studies conducted in Western countries.[13] See et al found that patients who were higher educated and held white collar occupations were more likely to have used CAM.[9]

It is difficult to make general comments regarding these findings about CAM use and sociodemographic variables, although gender does not seem an important factor and there are contradictory results about marital status among dermatology patients, but education level seems an important factor.

Although people percieve herbal remedies safe because they are natural, it is well known that they are not always innocent and may cause serious side effects. Bhuchar et al have underlined that "ingestible substances including most homeopatik, Ayurvedic and traditional Chinese medicine formulations that are not US FDA approved should be viewed with caution as they may cause severe adverse effects such as arsenicosis and hepatotoxicity" in their overview.[10] In Kutlu et al study 20.6 % of CAM users had been stated that they had experienced side effects.[15] It is difficult to analayze and make a comment about side effects regarding any specific CAM method as it is out of scope of this study. Sources of information on CAM have been usually other than health professionals and previous studies have shown that patients have not disclosed CAM use to their doctors unless it has been asked specifically. This may increase the risk of adverse effects and drug interactions due to inaccurate information and lack of medical fallow-up.

In our study, most commonly stated reason of not to diclose CAM use to the doctor was that it had been asked by the doctor; it was consistent with the findings of previous studies. These findings stress the need for doctors to inquire about CAM use of their patients.

Benefit from CAM use was at moderate or lower level in our study and few patients stated that they got benefit from the method they had used. In Kutlu et al study 64.2% of CAM users had thought the method they had used was ineffective.[25] Other studies have shown the similar findings. We cannot conclude that CAM use is ineffective as most of the patients have used CAM by theirselves without knowlegde of their doctors; so it is not possible to be sure that they have used CAM with right indication in the right way. On the other hand, dermatology patients indicated demand for having CAM approaches incorporated into their tratement reccomendations.[17,18]

The results of this study should be interpreted with caution due to limitations. The sample of the study is hospital based; not population-based which may differ regarding results. Also, it is not represantative at the national level which is especially important in a big country as Turkey having a very colourful cultural mosaic. Some of specific disease sub-group subjects were so few in the number and most ot the patients did not stated their diagnosis which may have effected the results regarding CAM use according to diagnosis. The results may not be generalized to the patients with other diseases than dermatology patients.

Conclusion

CAM use is a common practice among dermatology patients in Turkey. Patients who are higher educated, have longer disease duration or are suffering acne vulgaris or psoriasis are more likely to use

CAM. Taking into consideration of high prevalence of CAM use; dermatologists and primary care doctors should inquire about CAM use of their dermatology patients for preventing possible side effects and drug inreactions.

References

1. *nccam.nih.gov [Internet]. National Center for Complementary and Alternative Medicine. What is complementary and Alternative medicine? [updated 2011 july; cited 2008 oct]. Available from: http: //www.nccam.nih.gov/health/whatiscam/*
2. *Eisenberg DM, Kessler RC, Foster C, et al. Unconventional Medicine in the United states. The New England Journal of Medicine 1993; 328: 246-52.*
3. *Eisenberg DM, Davis RB, Ettner SL, et al. Trend in alternative medicine use in the United States. 1990-1997: Results of a fallow-up national survey. J Am Med Assoc 1998; 280: 1569-75.*
4. *Ernst E. Prevalence of use of complementary and alternative medicine (CAM): a systematic review. Bull World Health Organ 2000; 78: 252-7.*
5. *Harris P, Rees R. The prevalence of complementary and alternative medicine use among the general population: a systematic review of the literature. Complement Ther Med 2000; 8: 88-96.*
6. *Fuhrmann T, Smith N, Tausk F. Use of complementary and alternative medicine among adults with skin disease: updated results from a national survey. J Am Acad Dermatol. 2010; 63: 1000-5.*
7. *Kim GW, Park JM, Chin HW, et al. Comparative analysis of the use of complementary and alternative medicine by Korean patients with androgenetic alopecia, atopic dermatitis and psoriasis. J Eur Acad Dermatol Venereol [Internet]. 2012 May [Cited 2012 May 23]; doi: 10.1111/j.1468-3083.2012.04583.x [about one p.]. Available from: http: //www.ncbi.nlm.nih.gov/pubmed/22620682*
8. *Kalaaji AN, Wahner-Roedler DL, Sood A, et al. Use of complementary and alternative medicine by patients seen at the dermatology department of a tertiary care center. Complement Ther Clin Pract 2012; 18: 49-53.*
9. *See A, Teo B, Kwan R, et al. Use of complementary and alternative medicine among dermatology outpatients in Singapore. Australas J Dermatol 2011; 52: 7-13.*
10. *Bhuchar S, Katta R, Wolf J. Complementary and Alternative Medicine in Dermatology: An Overview of Selected Modalities for the Practicing Dermatologist. Am J Clin Dermatol 2012; 13: 311-7*
11. *Münstedt K, Harren H, Georgi R, Hackethal A. Complementary and Alternative Medicine: Comparison of current knowledge, attitudes and interest among German medical students and doctors. Evid Based Complement Alternat Med [Internet]. 2011 June[Cited 2011 June 18]; 2011(790951) [about one p.]. Available from: http: //www.ncbi.nlm.nih.gov/pmc/articles/PMC3153080/?tool=pubmed*
12. *Ernst E. The usage of complementary therapies by dermatological patients: a systematic review. Br J Dermatol 2000; 142: 857-61.*
13. *Chen YF, Chang JS. Complementary and alternative medicine use among patients attending a hospital dermatology clinic in Taiwan. Int J Dermatol 2003; 42: 616-21.*
14. *Gönül M, Gül U, Cakmak SK, Kiliç S. Unconventional medicine in dermatology outpatients in Turkey. Int J Dermatol 2009; 48: 639-44.*
15. *Kutlu S, Ekmekçi TR, Köşlü A, Pürisa S. Dermatoloji polikliniğine başvuran olgularda tamamlayici ve alternatif tip yöntemlerinin kullanimi[Use of complementary and alternative methods among patients admitted to dermatology clinic]. Turkiye Klinikleri J Med Sci 2009; 29: 1496-502*
16. *Fleischer AB, Feldman SR, Rapp SR, et al. Alternative therapies commonly used within a population of patients with psoriasis.Cutis 1996; 58: 2120.*
17. *Clark CM, McKay RA, Fortune DG, Griffiths CEM. Use of alternative treatments by patients with psoriasis. Br J Gen Pract 1998; 48: 1873–74.*
18. *Bilgiç Ö, Ak M. Akne vulgaris'li hastalarda tamamlayici ve alternatif tip uygulamalari [Complementary and alternative medicine use among patients with acne vulgaris]. İnönü Üniversitesi Tip Fakültesi Dergisi 2011; 18: 111-4.*
19. *Akan H, İzbirak G, Kaspar ÇE, et al. Knowledge and attitudes towards complementary and alternative medicine among medical students in Turkey. BMC Complement Altern Med. [Internet]. 2012 Aug [Cited 2012 Aug 03]; 12(115): [about one p.]. Available from: http: //www.biomedcentral.com/content/pdf/1472-6882-12-115.pdf*

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Acetabular component position of the noncemented total hip endoprosthesis after previous Chiari pelvic osteotomy

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Abstract

Introduction: The aim of the study was to determine the validity of acetabular component position of the noncemented total hip endoprosthesis after Chiari pelvic osteotomy.

Material and methods: The study involved 75 patients operated on at the Institute of Orthopedic Surgery "Banjica" in the period from 1990-2009. The first group consisted of 39 patients (46 hips) who underwent Chiari pelvic osteotomy and also later the implantation of a noncemented total hip endoprosthesis. A control group consisted of 36 patients (47 hips) who underwent total hip arthroplasty due to degenerative hip dysplasia.

Results: In the previously operated patients the centre of rotation of the hip was on the average placed more proximally, while in the control group of patients the position of the acetabular component was closer to the anatomical one. In the group of patients after Chiari osteotomy the mean acetabular cup abduction angle rated 41.8°±9.8°, while in the control group this value was on the average higher (45.4°±8.6°).

Discussion: There was a significant difference between the studied groups in relation to the distance between the acetabular component of endoprosthesis and the acetabular teardrop (t=-2.763; p=0.007). No statistically significant difference was determined in the mean value of the angle of acetabular abduction component of endoprosthesis between the studied groups of patients (t=1.878; p=0.064).

Conclusions: Acetabular component position of the total hip endoprosthesis was not compromised by anatomic changes of the acetabulum caused by Chiari pelvic osteotomy.

Key words: Chiari pelvic osteotomy, acetabular component position, total hip arthroplasty.

Introduction

The adequate acetabular component position can be a highly demanding procedure in cases of altered anatomic relationships that are to a lesser or higher degree present in patients with congenital hip dysplasia [1,2].

During surgical procedure of total hip endoprosthesis implantation it is our goal to place the acetabular component into a bearing created at the site of the true acetabulum. If this position does not render sufficient implant coverage to provide its stability and longevity, the use of acetabular augmentation is recommended [3,4,5]. Patients with congenital hip dysplasia may not have sufficient bone tissue at the site of the true acetabulum to provide a good support and coverage of the implant. In such cases one is forced to position the acetabular component at the site with sufficient bone tissue quantity, most often superiorly and laterally in relation to the position of the true acetabulum. Laterally and/or superiorly dislocated center of rotation of the hip increases the longitudinal loading of the acetabular component during activities, which accelerates insert wear and shortens endoprosthetic longevity [3,6,7].

It has been revealed that the acetabular component position of endoprosthesis can significantly influence the longitudinal loading that is transferred onto the insert thus thinning it with time [7]. The recommended position of the acetabular component is from 30°-50° of abduction from the horizontal plane [8].

Femoral head medialization by Chiari pelvic osteotomy was a frequently used procedure in the second half of the 20th century. Chiari surgery is nowadays recommended in patients with insufficient femoral head coverage when a full reconstruction of anatomic correlations cannot be ex-

pected even by utilization of other more efficient and technically more challenging pelvic osteotomies [9,10]. It is desirable that the patient has good strength of abductor musculature, preserved hip joint mobility, as well as absence of advanced degenerative disorders. A technically precisely performed Chiari osteotomy can result in the patient's good clinical and radiographic outcome over the next postoperative 10-20 years [11]. Despite improvement of joint congruency, degenerative disorders of the hip joint develop over time, so that the implantation of total hip endoprosthesis becomes inevitable (Figures 1, 2).

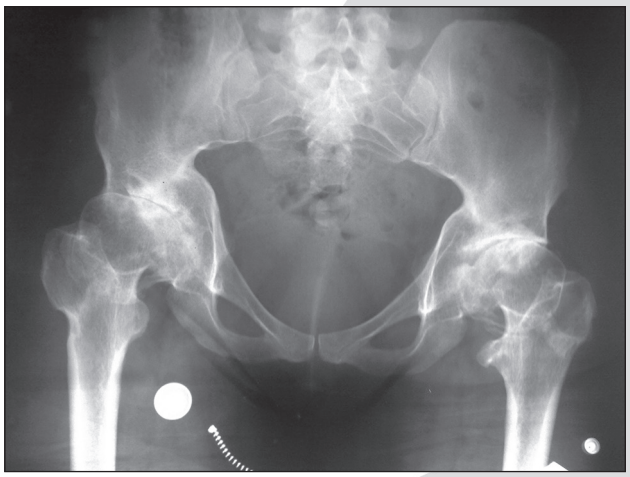

Figure 1. Severe hip arthrosis 15 years after bilateral Chiari osteotomies

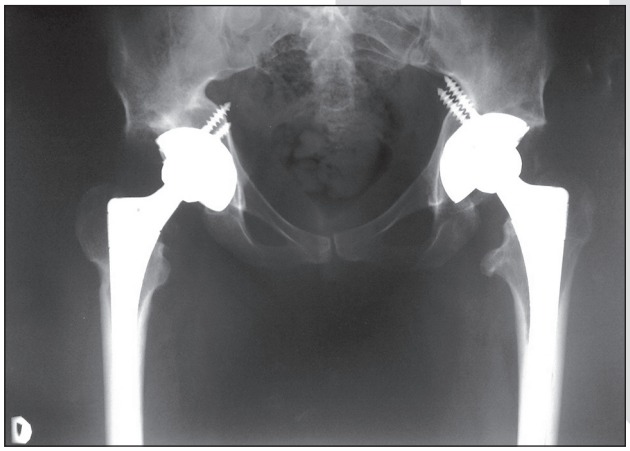

Figure 2. Bilateral total hip arthroplasty performed 16 years after Chiari pelvic osteotomies Acetabular cup positions are in the „safe zone" as described by Lewinnek with HHS above 90 bilaterally. Follow-up period of 4 years and 9 months after last surgery

The aim of the study was to determine the validity of acetabular component position of the noncemented total hip endoprosthesis after Chiari pelvic osteotomy, as well as the purposefulness of bone graft usage that could additionally improve this position.

Material and methods

The study included 75 patients (97 hips) with implanted noncemented total hip endoprosthesis due to a diagnosed degenerative disease. Thirty-nine patients (52%), i.e. 46 hips (49.5%) previously underwent Chiari pelvic osteotomy because of insufficient femoral head coverage. The control group was composed of 36 patients (48%), i.e. 47 hips (50.5%), in whom the degenerative disease also developed as the consequence of insufficient femoral head coverage but where no attempt was made to improve anatomic correlations by surgery. All total hip arthroplasties were performed at the Institute of Orthopedic Surgery (IOS) "Banjica" in the period from 1990-2009. In the study we used only complete and with fully conducted follow-up medical records and radiographies of the pelvis and hips in antero-posterior direction in standing position. In the evaluation of patients' functional status Harris Hip score was applied [11].

Immediately after the Chiari procedure, using radiographies of the pelvis with hips in the antero-posterior direction, we also measured and calculated osteotomy parameters: osteotomy angle, percentage of medial displacement of inferior pelvic fragment at the level of the osteotomy and the distance of the osteotomy from the acetabular edge. After performed total hip arthroplasty, the acetabular component abduction angle was measured, as well as the distance between its distal edge and the acetabular teardrop.

In all Chiari pelvic osteotomies the original surgical technique was respected [13]. In nine patients (19.6%) the occurrence of postoperative complications were recorded. The most frequent problem presented hardware breakage (55.6%), while osteotomy non-union, femoral head avascular necrosis, wound healing delay and joint contracture were equally represented (11.1%).

All surgical implantations of noncemented total hip endoprosthesis were performed via a pos-

terolateral approach. We used various primary implant models with noncemented fixation available in a corresponding time period.

All observed and calculated parameters were used in the formation of database and further statistical analysis. Descriptive statistical methods and methods for statistical hypothesis testing were used in the analysis of primary data. Statistical hypothesis testing involved the use of t-test, χ^2 test, Fisher test of exact probability, Wilks'Lambda test and variance of repeated measurements analysis (ANOVA). Statistical hypotheses were tested at the level of statistical significance of 0.05

Results

Of 93 operated hips only one (1.1%) belonged to a male patient who had previously undergone Chiari pelvic osteotomy. In the study lower extremities were approximately equally represented (47 right, 46 left), while arthroplasty was performed bilaterally in 18 patients (in 7 patients after Chiari osteotomy and in 11 control group patients). There was no statistically significant difference in the frequency of bilateral incidence of lower extremities between the two studied groups (χ^2=0.529; p=0.467).

The mean patients' age at the time of performed Chiari pelvic osteotomy was 37±9.4 years. The youngest patient was aged 15 years and the oldest one 52 years. The mean pelvic osteotomy angle was 10.3±8.6 degrees. In five (10.9%) patients osteotomy was performed completely horizontally (0°), while in one case the maximal determined value was 38 degrees. The mean value of femoral head medialization obtained after Chiari procedure rated 41.5±11.3%. The lowest recorded medialization was 20% and the highest 66.7%. The mean distance from the osteotomy site to the acetabular edge was 10.5±5.8 mm. The greatest measured distance of 26 mm was present in one patient. During triple surgeries (6.5%) osteotomy was initiated at the very acetabular edge through the joint capsule. No case of joint penetration was recorded.

The calculated mean time elapsed from the Chiari procedure to the implantation of total hip endoprosthesis was 194.2±75.8 months, ranging from 48 to 423 months.

The observed mean patients' age at the time of arthroplasty was 54.6±8.5 years; it rated 53.4±9.5 years in patients who had previously undergone Chiari pelvic osteotomy, i.e. 55.7±7.3 months in the control group patients. By statistical analysis it was determined that there was no significant difference in the mean patients' age at the time of total hip endoprosthesis implantation between the studied groups (t=1.306; p=0.195).

Femoral head autografts were used in 18 (19.35%) arthroplasties; of these, in 8 hips after Chiari pelvic osteotomy (17.4%), i.e. in 10 control group hips of previously unoperated patients (21.3%). No significant difference was determined in the frequency of autograft utilization between the studied groups (χ^2=0.225; p=0.635).

During 43 operations (19 times in patients who had previously undergone Chiari procedure, 24 times in previously unoperated patients), the acetabular component of the total hip endoprosthesis was placed at the site of true anatomic position. In 53.8% cases (27 times in patients after Chiari procedure and 23 times in control group patients) the center of rotation of the hip migrated more often proximally in relation to the teardrop (total 47 hips) than distally (in 3 patients previously unoperated by Chiari osteotomy). No statistically significant difference was found between the studied groups in relation to the position of the acetabular component to the acetabular teardrop (χ^2=0.891; p=0.345). In 10 cases (55.6%) the use of bone autograft resulted in the anatomically desired position of the acetabular component, but statistical significance of its utilization was not confirmed (χ^2=0.780; p=0.377).

The mean distance from the acetabular component distal edge to the acetabular teardrop was 7.1±9.6 mm. In 18 cases the use of bone graft decreased this distance to 4.7±7.7 mm, but without statistical significance (t=1.158; p= 0.250). In the patients previously operated on by the Chiari method the center of rotation of the hip was on the average positioned more proximally (9.8±11.1 mm; the most proximal position was measured 43 mm from the acetabular teardrop), while in the control group of patients acetabular component position was closer to the anatomic one (4.5±6.9 mm; ranging from 3 mm below the acetabular teardrop to 24 mm above the acetabular teardrop). There was a significant difference between the studied groups in relation to the distance between the acetabular component of the endoprosthesis and the acetabular teardrop

(t=-2.763; p= 0.007). By using the bone autograft, the center of rotation of the hip was positioned even closer to that of the true acetabular position (5.9±7.1 mm in the patients previously operated on by the Chiari method, i.e. 3.8±8.5mm in the control group patients). There was no confirmed statistical significant difference in the frequency of autograft use and the distance from the acetabular component to the acetabular teardrop (t=1.055; p=0.297 in the group of patients operated on by the Chiari method, i.e. t=0.373; p=0.711 in the group of previously unoperated patients).

The mean value of the acetabular component abduction angle was 43.6°±9.3. In the group of patients previously operated by Chiari osteotomy the mean abduction angle of the acetabular component was 41.8°±9.8°, while in the control group it was on the average higher (45.4°±8.6°). There was no significant difference in the mean acetabular component abduction angle of endoprosthesis between the studied groups of patients (t=1.878; p=0.064). In 18 cases autograft use slightly decreased acetabular component abduction angle (43.3°±6.8°), but without statistical significance (t=0.130; p=0.897).

In all 93 patients the mean value of HHS before the implantation of hip endoprosthesis was 51.9±12.7, and after performed surgery and rehabilitation it was 83.0±11.1. In the patients who had previously undergone Chiari osteotomy even more increased improvement of the functional status was observed (from 46.5±12.0 preoperatively to 84.0±13.4 postoperatively). In the previously unoperated patients the mean value of HHS before hip endoprosthesis implantation was 57.2±11.1. Postoperative functional status after rehabilitation period was improved to mean values of 82.1±8.3. There was a statistically significant increase of postoperative HHS values in both patient groups (Wilks'Lambda=0.170; p<0.001). In the observed time period there was a statistically significant difference in the HHS values between the studied groups (F=5.663; p=0.019). There was a statistically significant interaction in the patients operated on by Chiari osteotomy and previously unoperated patients in relation to HHS values during the observed period (Wilks'Lambda=0.833; p<0.015).

After implantation of hip endoprosthesis the patients were under clinical follow-up for the mean 66.6±38.3 months. The shortest follow-up period was 36 and the longest 222 months.

Discussion

Surgical procedure of Chiari's capsular arthroplasty has been applied, more or less frequently, for over 50 years. In the up-to-date orthopaedic practice Chiari pelvic osteotomy is considered a good alternative to total arthroplasty in patients with dysplastic hips and poor joint incongruence, particularly in the adolescent period [9,14,15].

Insufficiently good anatomic correlations between the femoral head and acetabulum nevertheless lead to the development of degenerative hip diseases in these patients. It has been observed that the implantation of total endoprosthesis after a previously performed Chiari osteotomy increases with the a longer follow-up period (Ito – 9% after 20.3 years, Kotz – 40% after 32 years) [9,10]. Decreased possibility of sufficient quality and long-term clinical follow-up of patients operated on by Chiari osteotomy most probably represents the major reason of such a low number of articles dealing with the problems of delayed implantation of total hip endoprosthesis. The group of 39 patients, i.e. 46 hips treated at the IOS "Banjica" over the past 22 years represents the largest group among those we have found in the up-to-date literature.

The mean age of patients at the time of Chiari pelvic osteotomy in our series was 37.0 years, which is significantly higher in relation to other similar studies [9,16]. Such data is not surprising having in mind that only patients who underwent implantation of total hip endoprosthesis are presented. In the study, conducted at the IOS "Banjica" which involved 86 patients (99 hips) of adolescent age ranging from 10-19 years, no case of hip arthroplasty was observed after Chiari pelvic osteotomy [14].

By analysis of obtained results in our series, it can be observed that the osteotomy angle and the distance between the site of osteotomy initiation and acetabular edge was wider, while the percentage of medialization was lower if compared to similar studies conducted in adolescent patients [14,17]. Those are exactly these differences that can explain different anatomic characteristics of bone and soft tissues among different age groups (thicker joint capsule, stronger and less elastic pelvic ring).

In our study the mean time between Chiari pelvic osteotomy and implantation of total hip endoprosthesis was 16.2 years. Ito et al. reported practically identical data (16.4 years), while other authors reported a considerably longer time period (Ohashi at al. 25 years, Kotz at al. 26 years) [9,10,11]. Nevertheless, a delay in exchanging the biological joint with an artificial one of over 15 years should not be considered failure, particularly if Chiari's capsular arthroplasty is indeed utilized as the salvage procedure in cases where a significant anatomic improvement by the use of some other pelvic osteotomy is not expected.

The mean age at the time of total hip arthroplasty was nor statistically significantly different between the two groups of patients. On this basis it is possible to conclude that Chiari osteotomy considerably prolongs the time of biological hip longevity and that, if performed correctly and timely, it enables a relatively favorable outcome in patients with insufficient femoral head coverage. Such a conclusion is of major significance, particularly if also kept in mind the patient's poor functional status immediately prior to performed Chiari pelvic osteotomy (HHS = 62.6±14.2).

Bulk autografts are often used in arthroplastic surgery of the hip with the basic goal of providing a good and reliable bearing for the acetabular component of endoprosthesis. Numerous authors insist on the reconstruction of the anatomic centre of rotation of the hip, with or without autograft, which decreases the possibility of accelerated polyethylene and alumina ceramic insert wear and the consequent implant loosening [1,4,5,6,18,19]. Georgiades et al. has proved increased frequency of loosening of the femoral component of endoprosthesis if the acetabular cup is placed more proximally than 25 mm in relation to the acetabular teardrop [2]. Bicanic et al. reported that longitudinal load on the acetabular cup increases by 0.1% for each millimeter of its proximal migration in relation to the position of the true acetabulum [7]. Schofer et al. consider that the autograft transplant and later loosening can be expected in approximately 50% of cases after 11.8-year period. The authors consider that the reconstruction of the anatomic rotation centre of the hip, bone quality, graft orientation and screws used for its fixation are the main factors which provide longevity of the noncemented acetabular component of total hip endoprosthesis positioned with the aid of autograft [3]. Kim et al. insist on the achievement of the primary initial stability of the implant and the use of a component of the largest possible diameter [4].

It should not to be discarded that there is a number of published papers by authors reporting the opinion that the use of bone autografts in dysplastic degeneratively altered hips is not justified [4,20,21]. Positioning the centre of hip rotation more proximally in relation to the true anatomic position (Kim et al. defines the position of high hip centre as the position that is over 35 mm more proximal in relation to the acetabular teardrop) is considered a more favorable and permanent solution if primary stability and sufficient bone coverage of the implant can be achieved [4]. Wan et al. report that the distance between the acetabular component and acetabular teardrop has no influence on the wear speed of polyethylene and ceramic inserts of hip endoprosthesis [22].

In our series, bulk structural bone autografts were used in approximately 20% patients, more or less identically in both groups of patients. In this way the anatomically desired position of the acetabular component was achieved in over 50% of cases (Figure 3). Nevertheless, according to statistical analysis the use of bone grafts in order to place the acetabular cup into the bearing created at the site of the true acetabulum is not justified. Statistical analysis indicates that by increasing the frequency of autograft utilization such a conclusion could be different and in accordance with the reports in the literature [1,3,4,5,6,19,23]. In our study, neither autograft resorption nor loosening of the acetabular cup placed in close proximity to the bone graft was recorded. Regarding the position of the acetabular component of endoprosthesis in relation to the acetabular teardrop, there was no statistically confirmed significant difference between the two groups. Nevertheless, it was noted that a slightly higher number of anatomically precisely positioned implants was present in the group of previously unoperated patients. The mean distance between the acetabular component distal edge and the acetabular teardrop was considerably greater in the patients operated by Chiari pelvic osteotomy (9.8 mm). In the control group of patients this distance was 4.5 mm, which is con-

siderably lower compared to the data published in the literature [4,18,21,24]. In a study, Fousek J. recorded that in patients with a degenerative disease, following a congenital disorder of the hips, the acetabular component was on the average positioned by 15 mm more proximally in relation to the acetabular teardrop [25].

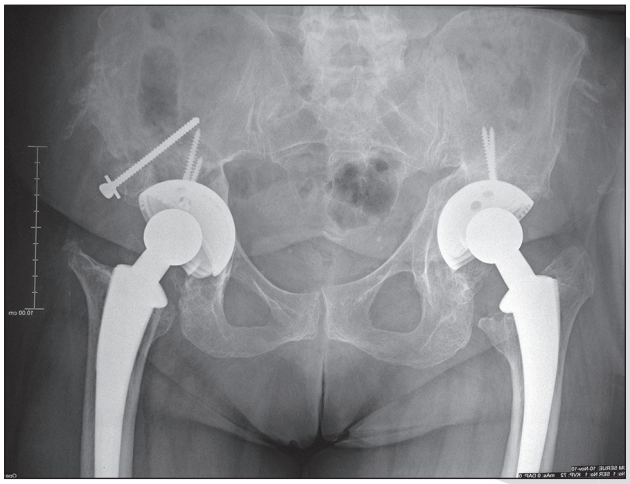

Figure 3. Total hip arthroplasty performed 15 years after Chiari pelvic osteotomies. Bulk femoral head autograft was used during right hip arthroplasty. Acetabular cups positions are without any significant differences. HHS=90, follow–up period of 7 years

Undoubtedly, acetabular anatomy, after Chiari pelvic osteotomy, can be the aggravating factor in the proper positioning of the acetabular component of hip endoprosthesis (Figures 4, 5).

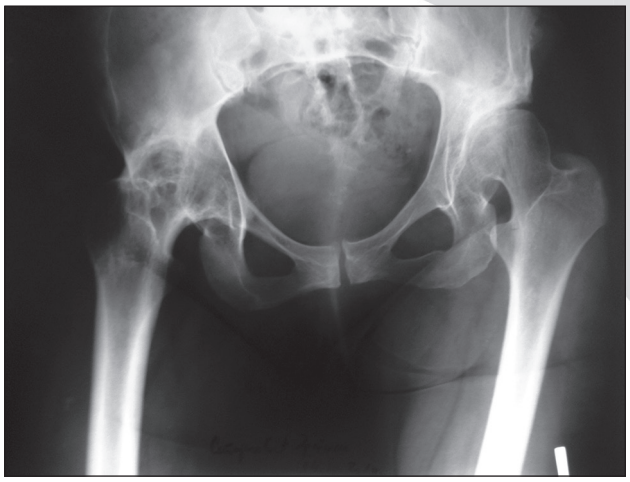

Figure 4. Left hip osteoarthrosis 24 years after Chiari pelvic osteotomy. Right hip arthrodesis is evident

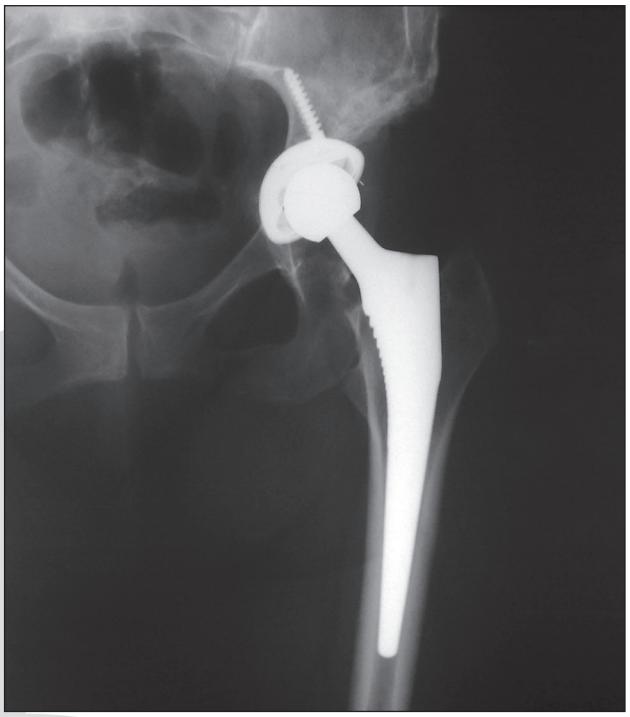

Figure 5. Total hip arthroplasty of the left hip. Medial cotyloplasty of the acetabulum was performed. Follow-up period of 3 years with HHS=83

Over 30 years ago Lewinnek identified a "safe zone" in positioning of the acetabular component of total hip endoprosthesis in order to lower the risk of postoperative hip dislocation [8]. Numerous papers have confirmed the original hypothesis according to which it should be insisted that the acetabular cup is to be placed in 40°±10° abduction and 15°±10° anteversion [26, 27]. According to the recent reports, abduction of over 45° is considered responsible for increased wear of polyethylene inserts [2, 22, 27]. In patients included in our study, the acetabular component was positioned by free-hand technique. The calculated values of abduction implant did not significantly differ between the two groups of patients and were positioned within the recommended range. These results are in accordance with similar studies [28]. It is interesting to mention that in 18 cases the use of autograft did not significantly alter the values of the acetabular component abduction of endoprosthesis. The altered acetabular anatomy has more influence on the height of the acetabular cup in relation to the teardrop than on its abduction.

According to most authors, altered acetabular anatomy after Chiari pelvic osteotomy does not represent a significant problem in arthroplastic joint

reconstruction surgery [11, 29, 30]. Based on 10 implanted hip endoprostheses, Minoda et al. reports that the recorded prolonged time of surgery involves higher intraoperative blood losses as well as a poorer position of acetabular components [31].

By the implantation of total hip endoprosthesis and later rehabilitation, the functional status of patients with degenerative disease of the dysplastic hip is considerably improved. Preoperative and postoperative values obtained in our study are within the range recorded in the up-to-date literature dealing with similar topic [19, 23, 24]. Johansson et al. points out that, after the implantation of total hip endoprosthesis, better functional results have been achieved in patients with a higher preoperative HHS [32]. The author suggests a timely surgical intervention so as to provide best possible postoperative function. Results published by Fousek et al. are contrary to the aforementioned data [25]. According to his study, after the implantation of total hip endoprosthesis patients with poorer preoperative functional status had higher HHS values (HHS=38.6 preoperatively, 80.3 postoperatively in group I; HHS=35.5 preoperatively, 84.9 postoperatively in group II). Similar results were also recorded by analysis and processing of our data. After the implantation of total hip endoprosthesis the patients had a considerably higher functional improvement (HHS from 46.5 preoperatively to 84.0 postoperatively) in relation to the control group of previously unoperated patients (HHS from 57.2 preoperatively to 82.1 postoperatively). Such results confirm the significance of technically properly performed arthroplasty, by which, despite preoperative anatomic relations of different complexity, a practically equally favorable postoperative outcome can be achieved. In the process of making conclusions one should not leave out the fact that the preoperative functional status of patients operated on by the Chiari method was considerably inferior in relation to the group of patients who had not previously undergone surgical treatment.

Conclusion

Chiari pelvic osteotomy postpones degenerative processes in patients with insufficient femoral head coverage but it also considerably changes anatomy of the acetabulum. Positioning of the acetabular component during a delayed arthroplasty is not compromised by such anatomic changes. The use of bulk bone autographs decreases the distance between the centre of rotation of the hip and the acetabular teardrop, but it does not have influence on the abduction of the acetabular cup.

Acknowledgement

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References

1. *Dapuzzo MR, Sierra RJ. Acetabular considerations during total hip arthroplasty for hip dysplasia. Orthop Clin North Am. 2012; 43(3): 369-75.*
2. *Georgiades G, Babis GC, Kourlaba G, Hartofilakidis G. Effect of cementless acetabular component orientation, position, and containment in total hip arthroplasty for congenital hip disease. J Arthroplasty. 2010; 25(7): 1143-50.*
3. *Schofer MD, Pressel T, Schmitt J, et al. Reconstruction of the acetabulum in THA using femoral head autografts in developmental dysplasia of the hip. J Orthop Surg Res. 2011; 6: 32.*
4. *Kim M, Kadowaki T. High long-term survival of bulk femoral head autograft for acetabular reconstruction in cementless THA for developmental hip dysplasia. Clin Orthop Relat Res. 2010; 468(6): 1611-20.*
5. *Shetty AA, Sharma P, Singh S, et al. Bulk femoral-head autografting in uncemented total hip arthroplasty for acetabular dysplasia: results at 8 to 11 years follow-up. J Arthroplasty. 2004; 19(6): 706-13.*
6. *Yang S, Cui Q. Total hip arthroplasty in developmental dysplasia of the hip: Review of anatomy, techniques and outcomes. World J Orthop. 2012; 3(5): 42-8.*
7. *Bicanic G, Delimar D, Delimar M, Pecina M. Influence of the acetabular cup position on hip load during arthroplasty in hip dysplasia. Int Orthop. 2009; 33(2): 397-402.*
8. *Lewinnek GE, Lewis JL, Tarr R, et al. Dislocations after total hip-replacement arthroplasties. J Bone Joint Surg Am. 1978; 60(2): 217-20.*
9. *Ito H, Tanino H, Yamanaka Y, et al. The Chiari pelvic osteotomy for patients with dysplastic hips and poor joint congruency: long-term follow-up. J Bone Joint Surg Br. 2011; 93(6): 726-31.*

10. *Kotz R, Chiari C, Hofstaetter JG, et al. Long-term experience with Chiari's osteotomy. Clin Orthop Relat Res. 2009; 467(9): 2215-20.*

11. *Ohashi H, Hirohashi K, Yamano Y. Factors influencing the outcome of Chiari pelvic osteotomy: a long-term follow-up. J Bone Joint Surg Br. 2000; 82(4): 517-25.*

12. *Harris WH. Traumatic arthritis of the hip after dislocation and acetabular fractures: treatment by mold arthroplasty. An end-result study using a new method of result evaluation. J Bone Joint Surg Am. 1969; 51(4): 737-55.*

13. *Chiari K. Medial displacement osteotomy of the pelvis. Clin Orthop Relat Res. 1974; (98): 55-71.*

14. *Vukašinović Z, Spasovski D, Slavković N, et al. Chiari pelvic osteotomy in the treatment of adolescent hip disorders: possibilities, limitations and complications. Int Orthop. 2011; 35(8): 1203-8.*

15. *Ito H, Matsuno T, Minami A. Chiari pelvic osteotomy for advanced osteoarthritis in patients with hip dysplasia. J Bone Joint Surg Am. 2005; 87 Suppl 1(Pt 2): 213-25.*

16. *Gagała J, Blacha J, Bednarek A. Chiari pelvic osteotomy in the treatment of hip dysplasia in adults. Chir Narzadow Ruchu Ortop Pol. 2006; 71(3): 183-5.*

17. *Karami M, Fitoussi F, Ilharreborde B, et al. The results of Chiari pelvic osteotomy in adolescents with a brief literature review. J Child Orthop. 2008; 2(1): 63-8.*

18. *Flecher X, Parratte S, Brassart N, et al. Evaluation of the hip center in total hip arthroplasty for old developmental dysplasia. J Arthroplasty. 2008; 23(8): 1189-96.*

19. *Ermiş MN, Dilaveroğlu B, Erçeltik O, et al. Intermediate-term results after uncemented total hip arthroplasty for the treatment of developmental dysplasia of the hip. Eklem Hastalik Cerrahisi. 2010; 21(1): 15-22.*

20. *Holzapfel BM, Greimel F, Prodinger PM, et al. Total hip replacement in developmental dysplasia using an oval-shaped cementless press-fit cup. Int Orthop. 2012; 36(7): 1355-61.*

21. *Kaneuji A, Sugimori T, Ichiseki T, et al. Minimum ten-year results of a porous acetabular component for Crowe I to III hip dysplasia using an elevated hip center. J Arthroplasty. 2009; 24(2): 187-94.*

22. *Wan Z, Boutary M, Dorr LD. The influence of acetabular component position on wear in total hip arthroplasty. J Arthroplasty. 2008; 23(1): 51-6.*

23. *Wu LD, Jin LB, Yan SG, et al. Total hip arthroplasty with cementless cups and femoral head autografts for patients with hip dysplasia and osteoarthritis. Chin J Traumatol. 2004; 7(5): 280-5.*

24. *Murayama T, Ohnishi H, Okabe S, et al. 15-year comparison of cementless total hip arthroplasty with anatomical or high cup placement for Crowe I to III hip dysplasia. Orthopedics. 2012; 35(3): e313-8.*

25. *Fousek J, Indráková P. Total hip arthroplasty in post-dysplastic hip arthritis. Can type and position of the acetabular component influence longevity of the prosthesis? Acta Chir Orthop Traumatol Cech. 2007; 74(1): 47-54.*

26. *Yoon YS, Hodgson AJ, Tonetti J, et al. Resolving inconsistencies in defining the target orientation for the acetabular cup angles in total hip arthroplasty. Clin Biomech (Bristol, Avon). 2008; 23(3): 253-9.*

27. *Epstein NJ, Woolson ST, Giori NJ. Acetabular component positioning using the transverse acetabular ligament: can you find it and does it help? Clin Orthop Relat Res. 2011; 469(2): 412-6.*

28. *Kumar JN, Kumar JS, Wang VT, Das De S. Medium-term outcome of total hip replacement for dysplastic hips in Singapore. J Orthop Surg (Hong Kong). 2010; 18(3): 296-302.*

29. *Hashemi-Nejad A, Haddad FS, Tong KM, et al. Does Chiari osteotomy compromise subsequent total hip arthroplasty? J Arthroplasty. 2002; 17(6): 731-9.*

30. *Tokunaga K, Aslam N, Zdero R, et al. Effect of prior Salter or Chiari osteotomy on THA with developmental hip dysplasia. Clin Orthop Relat Res. 2011; 469(1): 237-43.*

31. *Minoda Y, Kadowaki T, Kim M. Total hip arthroplasty of dysplastic hip after previous Chiari pelvic osteotomy. Arch Orthop Trauma Surg. 2006; 126(6): 394-400.*

32. *Johansson HR, Bergschmidt P, Skripitz R, et al. Impact of preoperative function on early postoperative outcome after total hip arthroplasty. J Orthop Surg (Hong Kong). 2010; 18(1): 6-10.*

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Morphological features of the third branch of the main trunk of the left coronary artery

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Abstract

This study examined the number of terminal branches of main trunk of left coronary artery, as well as the frequency of diagonal branch of the left coronary artery.

Coronary angiograms of 150 adult patients with coronary artery disease (100 males, 50 females; age range, 35–75 years) who underwent coronary angiography in Heart Centre of the Clinical Center University of Sarajevo in the last 2 years, were reviewed to identify the coronary anatomy and determine the existence of diagonal branches of the left coronary artery.

Our results reveal that the main trunk of left coronary artery terminates in a bifurcation into the anterior interventricular branch and the circumflex branch (56%), trifurcation into the anterior interventricular branch, circumflex branch and diagonal branch (40%), or occasionally quadrifurcation into the anterior interventricular branch, circumflex branch and two diagonal branches (4%). The diagonal branch derived from the left anterior descending artery were seen in 96% of cases. In 4% of cases (6 patients) the diagonal branch separated directly from the bifurcation of the left main stem (known as the "intermediate" and "median" artery). There were significant differences in the number of branches of the left coronary artery between groups.

Knowledge of variations in the angiographic anatomy of left coronary artery is of immense value during interpretation of coronary angiography procedures as well as for hemodynamic and surgical manipulation. The diagonal branch present important pattern of the collateral blood flow, which has special meaning under conditions of coronary insufficiency.

Key words: main trunk of left coronary artery, diagonal branch, coronary angiography

Introduction

The left coronary artery is larger in calibre than the right, and supplies a greater volume of myocardium. Patency of the left coronary artery is vital for sufficient perfusion of most of the heart. The left coronary artery is responsible for irrigation not only of most of the left ventricle, but also a considerable proportion of the right ventricle (1). The most frequent type of division of the left coronary artery is a bifurcation into two terminal branches: anterior interventricular and circumflex branches (2,3). In some cases the main trunk of left coronary artery divides into three terminal branches, trifurcates, producing anterior interventricular branch, circumflex branch and a diagonal branch, and tetrafurcates, producing anterior interventricular branch, circumflex branch and two diagonal branches (4,5,6,7,8). The terms "intermediate" or "median artery" should be used for the third branch of the left coronary artery originating from the main trunk of the left coronary artery between the circumflex branch and anterior interventricular branch. "Intermediate" and "median" refers to the origin but "diagonal" refers to the course of the artery. This branch, including its anastomoses, presents important pattern of the collateral blood flow, which has special meaning under conditions of coronary insufficiency (1,9,10,11,12). The main trunk of left coronary artery divided into three terminal branches Hadžiselimović (3) established in 52% dissected human hearts. In addition, the diagonal branches originating from anterior interventricular branch.

The aim of this study was to analyze the morphological characteristics of the branches of the main trunk of the left left coronary artery and to prove importance of the diagonal branch existence in the conditions of coronary insufficiency. In addition, we compared our results with reports performed with different methods previously (1-12).

Patients and Methods

Coronary angiograms of 150 adult patients with coronary artery disease (100 males, 50 females; age range, 35–75 years) who underwent coronary angiography in Heart Centre of the Clinical Center University of Sarajevo in period of the last 2 years, were reviewed to identify the coronary anatomy and determine the existence of diagonal branches of the left coronary.

Angiographically, the patients were divided into groups: the first group with bifurcation of the left coronary artery into the left anterior descending artery (LAD) and circumflex branch (Cx); the second group with trifurcation of the left coronary artery into left anterior descending artery, circumflex branch and diagonal branch (D), and the group with quadrifurcation of the left coronary artery into left anterior descending artery, circumflex branch and two diagonal branches.

The angiograms were analyzed to determinate the origin of diagonal branch of the left coronary artery.

Statistical analysis

The statistical analysis of the results was performed using Kolmogorow-Smirnow test and the differences in the prevalence of terminal branches of the left coronary artery between groups were considered significant on the level $p<0,05$.

Results

The coronary angiograms of 150 patients were reviewed and angiograms of 60 adult patients were identified with existence of the diagonal branch of the left coronary artery. The left coronary artery originated from the left sinus of Valsalvae in all cases. The most frequent type of division of the main trunk of left coronary artery was bifurcation into the left anterior descending artery and circumflex branch - 84 patients (56% of cases). (Figure 1, Table 1). Trifurcation of the left coronary artery into left anterior descending artery, circumflex branch and diagonal branch was established in 60 patients (40% of cases). Quadrifurcation of the left coronary artery into left anterior descending artery, circumflex branch and two diagonal branches was described in 4% of cases (6 patients) (Table 1). The diagonal branch derived from the left anterior descending artery (LAD) were seen in 96% of cases (Figure 2, Table 2). In 4% of cases (6 patients) the diagonal branch separated directly from the bifurcation of the left main stem (known as the "intermediate" and "median" artery) (Figure 3, Table 2). Kolmogorov-Smirnov test shows significant differences in the number of branches of the left coronary artery between groups. In patients with diagonal branch derived from the left anterior descending artery we establisched stenosis of LAD.

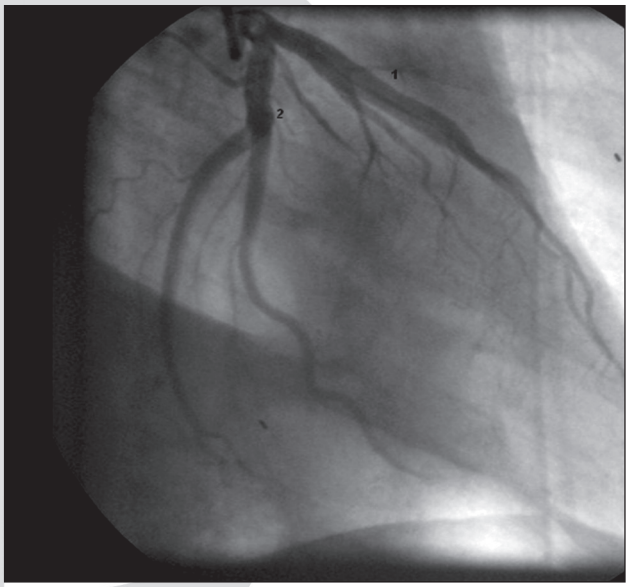

Figure 1. Bifurcation of the left coronary artery into the left anterior descending artery, LAD (1) and circumflex branch, Cx (2)

Table 1. The number of terminal branches of main trunk of the left coronary artery

Terminal branches of main trunk of the left coronary artery			
Bifurcation	Trifurcation	Quadrifurcation	Total (%)
84 (56%)	60 (40%)	6 (4%)	150 (100%)

Table 2. The origin of diagonal branch of the left coronary artery

Diagonal branch derived from the left anterior descending artery	Diagonal branch separated directly from the left main	Total (%)
144 (96%)	6 (4%)	150 (100%)

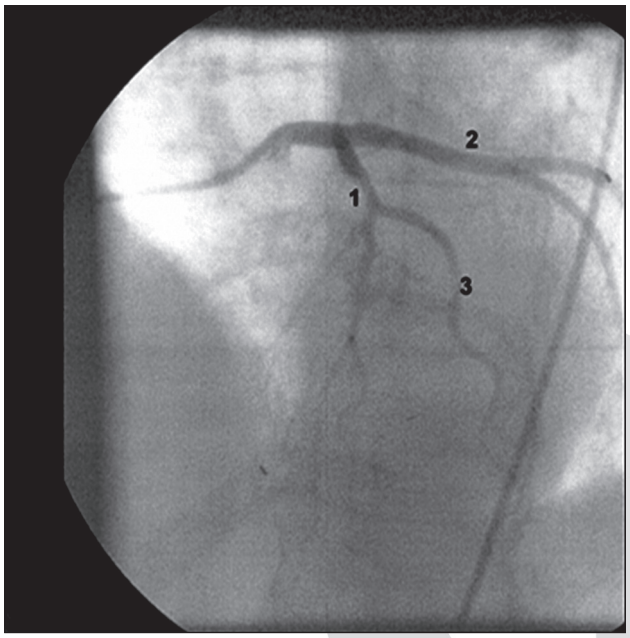

Figure 2. Coronary angiography in the left anterior oblique projection (LAO) showed the origin of diagonal artery (3) from left anterior descending artery with stenosis (1) and circumflex branch (2)

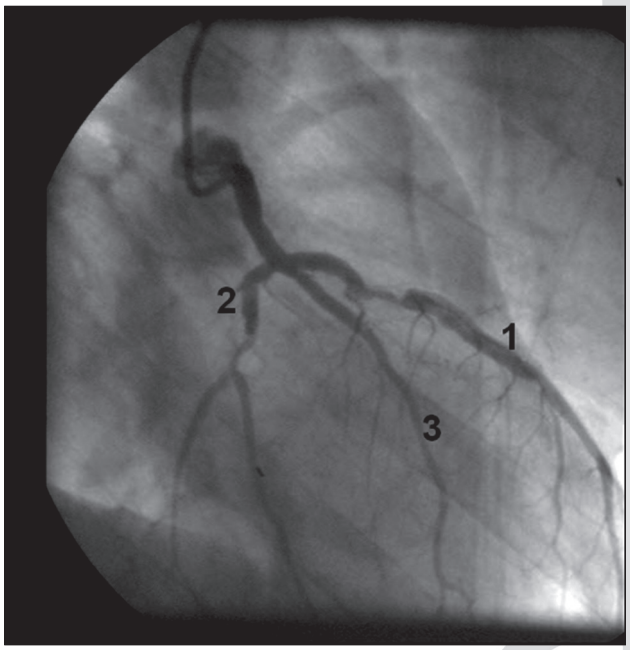

Figure 3. Coronary angiogram in the right anterior oblique projection (RAO) showed the origin of diagonal artery (3) from the main trunk of the left coronary artery between the circumflex branch (2) and left anterior descending artery (1)

Discussion

Branches of coronary arteries may vary in origin, distribution, number and size. Many researchers reported different results about the branching frequency of the left coronary artery, the length of the main trunk of the left coronary artery and the length of the median artery (1-12).

The left coronary artery trunk divides in several ways; it bifurcates, producing the anterior interventricular branch and the circumflex branch, trifurcates, producing the anterior interventricular branch, circumflex branch and a diagonal branch, and tetrafurcates, producing the anterior interventricular branch, circumflex branch and two diagonal branches (5).

Previous studies have reported wide variation in left coronary artery trunk branching and have found a greater prevalence of bifurcated expression. Our results showed bifurcation into the left anterior descending artery and circumflex branch in 84 patients (56% of cases), coincided with previous reports indicating 40–85% (1,2,3,4,5,7,11,12). The trifurcated division (with the addition of a diagonal branch) observed in this work in 60 patients (40%). Our results was in in relation to previous reports (3,4,5,7,11), although Kýlýç et al. (1) reported trifurcation in 14% of their cases, Kulkarnie et al. (8) discovered trifurcation in 11,54% of cases.

The frequency of left coronary artery trunk quadrifurcation into left anterior descending artery, circumflex branch and two diagonal branches in our study was described in 4% of cases (6 patients), similar to that reported by previous authors (5-11%).

Out of these 150 patients with signs of coronary insufficiency the diagonal branch separated from the left anterior descending artery were seen in 96% of cases. In 4% of cases (6 patients) the diagonal branch separated directly from from the bifurcation of the left main stem (known as the "intermediate" and "median" artery).

There were significant differences in the number of branches of the left coronary artery between groups.

While Vilallonga (9) reported that the external diameter of the median artery was thinner than the anterior interventricular branch and thicker than the circumflex branch, Surucu et al (2) reported that it was thinner than both. Our study was similar to Surucu et al's and Fazliogullari et al's (4), in that the average median artery thickness was lower than that of the anterior interventricular branch and the circumflex branch.

In some patients with diagonal branch derived from the left anterior descending artery we established stenosis of LAD. In patients with obstruction of LAD or CX diagonal branch present important pattern of the collateral blood flow. The diagonal branch as important pattern of the collateral blood flow has special meaning under conditions of coronary insufficiency.

The high degree of variability of the coronary arteries and their branches must be carefully observed and studied from anatomical, pathophysiological, diagnostic and therapeutic viewpoints. (5).

Knowledge of variations in the angiographic anatomy of left coronary artery is of immense value during interpretation of coronary angiography procedures as well as for hemodynamic and surgical manipulation.

Conclusions

The third branch of the main trunk of the left coronary artery was established in 40% of patients with sins of coronary insufficiency. The diagonal branch derived from the left anterior descending artery were seen in 96% of cases. In 4% of cases the diagonal branch separated directly from the bifurcation of the left main stem.

The diagonal branch present important pattern of the collateral blood flow, which has special meaning under conditions of coronary insufficiency. Knowledge of the morphological characteristics of the main trunk of the left coronary artery as well as its variations is essential for hemodynamic and surgical manipulation as well as for correctly interpreting angiographic data.

References

1. *Kýlýç C, Kýrýcý Y. Third branch derived from left coronary artery: the median artery, Gülhane Týp Dergisi 2007; 49: 232-235.*
2. *Surucu HS, Karahan ST, Tanyeli E. Branching pattern of the left coronary artery and an important branch. The median artery. Saudi Med J. 2004; 25(2): 177-81.*
3. *Hadžiselimović H. Krvni sudovi srca. Jugoslavenska medicinska naklada Zagreb, 1981.*
4. *Fazliogullari Z, Karabulut AK, Unver Dogan N, Uysal I I. Coronary artery variations and median artery in Turkish cadaver hearts. Singapore Med J 2010; 51(10): 775-80.*
5. *Ballesteros LE, Ramirez LM. Morphological expression of the left coronary artery: a direct anatomical study. Folia Morphol. 2008; 67(2): 135-142.*
6. *Bhimalli S, Dixita D, Siddibhavi M, Spirol VS. A study of variations in coronary arterial system in cadaveric human heart. World Journal of Science and Technology 2011; 1(5): 30-35.*
7. *Dombe DD, Anitha T, Giri PA, et al. Clinically relevant morphometric analysis of left coronary artery. Int J Biol Med Res. 2012; 3(1): 1327-1330.*
8. *Kulkarni JP, Mehta L. Main left coronary artery system – Angiographic Anatomy. Journal of Dental and Medical Sciences. 2012; 3(2): 05-07.*
9. *Vilallonga JR. Anatomical variations of the coronary arteries: I. The most frequent variations. Eur J Anat. 2003; 7(1): 29-41.*
10. *Das Hirak. Termination of left coronary artery in the population of Assam. National Journal of basic medical sciences. 2005; 1(3): 145-148.*
11. *Kaplana R. A Study on principal branches of coronary arteries in humans. J Anat. Soc. India, 2003; 52(2): 137-140.*
12. *Hasanović A, Junuzović A, Spužić M, Kudumović. Angiographic evaluation of myocardial bridges in relation to myocardial ischemia. HealthMED 2010; 4(2): 398-403.*

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Influences of chronic variable stress on levels of interleukin-17A, IFN-γ and TNF-α in OVA-induced asthmatic rats

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Abstract

Aims: The aims of the present study were to examine the influences of chronic variable stress (CVS) on levels of TH1 cytokines (IFN-γ and TNF-α) and TH17 cytokine interleukin-17A (IL-17A), and on the ultrastructure of type I pneumocytes in OVA-induced asthmatic rats.

Methods: Two groups of Wistar rats were sensitized and challenged with ovalbumin (OVA). Then, one group of the OVA-induced asthmatic rats received a chronic variable stress procedure lasting for 28 days. Levels of IL-17A, IFN-γ and TNF-α in BAL fluid and Plasma of the rats were examined using Elisa. Ultrastructural changes of type I pneumocytes were observed by electron microscopy.

Results: Higher levels of IL-17Aand TNF-α were observed in the OVA-induced asthmatic rats compared to the control rats, while IFN-γ levels were lower in the OVA-induced asthmatic rats than the control animals. CVS further increased IL-17A levels of the OVA-induced asthmatic rats, while levels of IFN-γ and TNF-α did not change when exposed to CVS. CVS also induced greater numbers of leukocytes in blood of the OVA-induced asthmatic rats. Type I pneumocytes of the rat in CVS group showed compaction, segregation and location on the nuclear envelope of the nuclear chromatin under the transmission electron microscope.

Conclusions: CVS could significantly increase IL-17A levels and induce cellular damage to type I pneumocytes of the OVA-induced asthmatic rats.

Key words: Asthma, stress, Interleukin-17A, IFN-γ, TNF-α, type I pneumocyte, ultrastructure

Introduction

Epidemiological studies have well demonstrated that increased asthma prevalence and asthma exacerbation are closely associated with psychological stress, such as depression [1-3]. A tremendous amount of attention has been paid to the mechanisms linking psychological stress and asthma exacerbation [4]. However, conclusive experimental evidence is limited so that the underlying mechanisms have not been well understood.

Allergic asthma is a chronic inflammatory respiratory disease characterized by a predominance of Th2 cells [5]. Animal studies show that Th2 cells are required for development of allergic airway inflammation, up-regulated systemic Th2 cytokine production can promote airway inflammation [6], airway obstruction [6], hyperresponsiveness [7] and airway remodeling [8], which all may cause asthma exacerbation. In contrast, Th1 cells had been regarded to inhibit bronchial asthma by production of IFN-γ [9]. It is generally accepted that patients with asthma usually have the problem of Th1/Th2 imbalance, namely over-expression of Th2 cells and Th2 cytokines together with suppression of Th1 cells and Th1 cytokines. Further, studies have indicated some psychological disorders, such as depression, as inflammatory state with altered levels of proinflammatory cytokines. A study showed that an imbalance between Th1 and Th2 cytokines (downregulation of IFN-gamma or TNF-alpha and upregulation of IL-4) occurred in untreated depressed patients[10]. In contrast, a study demonstrated that the Th1 and Th2 cytokines imbalance with increased level of INF-gamma was observed in depressed patients. [11]. Another recent study showed lower levels of Th2 cytokine IL-6 in people with symptoms of depression [12]. More interestingly, in a study by Gabbay V, et al, the authors concluded that an imbalance of Th1/Th2 shifted toward Th1 might be associated with adolescent major depressive disorder [13].

Thus, the alteration of Th1 and Th2 cytokines in cases with psychological disorders are still debated. Further, little is known about the influences of chronic psychological stress on inflammatory cytokines in asthmatic cases. We have previously detected the changes of Th2 cytokines interleukin-1β and interleukin-6 in a rat model of asthma with depressive-like behaviors [1]. In the present study, we examined levels of Th1 cytokines IFN-γ and TNF-α in asthmatic rats after exposure to CVS.

Though the balance of Th1/Th2 plays a central role in inflammatory diseases, more and more evidence showed its limitation in explaining the pathogenesis of the diseases. Attention also has been focus on the role of another type of T cells, Th17 cell, in inflammatory diseases [14-19]. Recent studies have demonstrated that Th17 cytokine IL-17 is involved in asthma by promoting airway inflammation, hyperresponsiveness and airway remodeling [20-25]. Further, levels of IL-17A in cases with psychological stress also alter and are important for the pathogenesis of psychological disorders [26, 27]. However, the influence of CVS on levels of IL-17A in asthmatic cases still remains unclear.

Asthmatic cases with psychological disorders usually have poorer lung function than the cases without psychological problems. It is accepted that psychological stress can exacerbate asthma by enhancing airway reactivity and promoting airway inflammation. However, it has not been well demonstrated whether the psychological stress produce adverse influence on type I pneumocyte that is important for maintaining normal lung function.

Thus, the present study sought to evaluate the influences of CVS on levels of Th1 cytokines (TNF-α and IIFN-γ) and Th17 cytokine (IL-17A) in OVA-induced asthmatic rats. In addition, we investigated the role of CVS on ultrastructure of type Ⅰ pneumocytes in asthmatic animals.

Materials and Methods

Animals

This study was approved by Weifang Medical University Animal Ethics Committee and was conducted in accordance with the Chinese Council of Animal Care guidelines and Use of Laboratory animals prepared by the Weifang Medical University, Animal Ethics Committee.

Male Wistar rats (200–220 g) were purchased from the animal center in Shandong University. Animals were maintained on a controlled 12 h: 12 h light-dark cycle with constant temperature and humidity. Rats were randomly divided into control group, asthma group and CVS group with 10 rats in every group.

OVA-Induced asthmatic rat model

Rats in asthma group and CVS group were sensitized to OVA by intraperitoneal injection of OVA–aluminum hydroxide dissolved in 1 ml of sterile saline on day 1 and day 7, then challenged by 1% OVA aerosol via airway from day 15 using a ultrasonic nebulizer. The OVA-challenge consisted of an acute phase and a chronic phase. Control rats were exposed to sterile saline using the same method, as described previously [1].

CVS exposure

One day after the acute OVA-challenge phase, rats in the CVS were subjected to the CVS procedure consisting of kinds of unpredictable stressors for a period of 28 consecutive days. Rats received one stressor in a random order per day, as described previously [1]. The rats in the control group received no stressors during the period.

Measurement of levels of interleukin-17A, IFN-γ and TNF-α

After the last stressor, one milliliter blood was collected centrifuged at 1500 rpm to get plasma under deep anesthesia. BAL fluid was collected as reported previously. The samples were stored at -80℃ for measurement of cytokines. The levels of IL-17A, IFN-γ and TNF-α in plasma and BLA fluid of the rats were determined according to the manufacturer's instructions using ELISA (R&D Systems, USA).

Transmission electron microscopy

To examine the ultrastructural changes of type I pneumocytes of the rats, Small tissue blocks of the left lung were collected, washing with 0.1 M phosphate buffer. The samples were put into 3% glutaraldehyde for 12h, followed by dehydration with graded ethanol solutions. Then the lung tissue was embedded in epoxy resin. Eighty nm thick sections were cut with an ultramicrotome,

mounted on copper grids, stained with uranyl acetate, and examined by transmission electron microscope operated at 60 kV.

Data analysis

Numerical data were expressed as means ± SE, and analyzed using SPSS 13.0. One-way ANOVA followed by Scheffe test was used to evaluate differences between groups. Significance was considered at $P < 0.05$.

Results

Inflammatory cells in blood

To determine whether chronic stress could exacerbate airway inflammation of asthma rats, we have examined numbers of inflammatory cells in BLA fluid in the previous study [1]. Further, in the present study, we examined numbers of inflammatory cells in blood of the asthma rats after CVS stimulation. Analysis of the leukocyte populations demonstrated that numbers of inflammatory cells including total leukocytes, eosinophils and lymphocytes in blood of the asthma rats increased compared to the control rats(all $p<0.05$). CVS further increased inflammatory cells in blood as compared with asthma rats without CVS stimulation ($p<0.05$). Thus, our data indicated that CVS stimulation exacerbated the inflammation of asthma rats. (Shown in table 1)

Levels of interleukin-17A, IFN-γ and TNF-α

Our previous study showed that chronic stress could promote the production of TH2 cytokines interleukin-1β and interleukin-6. In this study we examined levels of TH1 cytokines IFN-γ and TNF-α, TH17 cytokine TH17A. The data showed that asthma rats had higher levels of interleukin-17A and TNF-α both in BLA fluid and plasma (both $p<0.05$), while lower level of IFN-γ ($p<0.05$), as compared with the rats in control group. Rats in CVS group had higher levels of IL-17A than the asthma rats without CVS exposure in BLA fluid and plasma (both $p<0.05$), while levels of IFN-γ and TNF-α both in BLA fluid and plasma had no significant difference between the two groups ($p>0.05$). The results suggested that Th17 cytokines rather than Th1 cytokines were involved in the pathogenesis of asthma with comorbid psychological stress. (Shown in table 2)

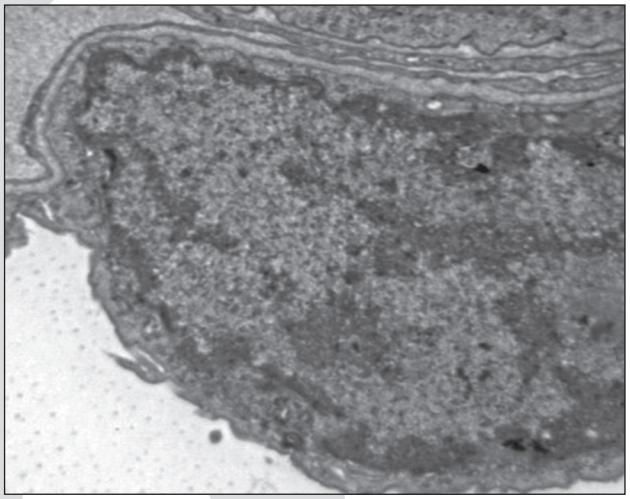

Figure 1. Type I pneumocyte f from the rat in CVS group

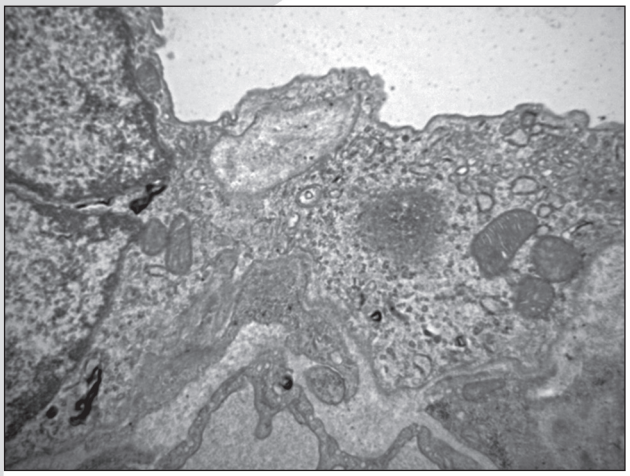

Figure 2. Type I pneumocyte from the rat in asthma group

Table 1. Inflammatory cells in blood in rats

Group	Total leukocytes ($\times 10^5$)	Eosinophils ($\times 10^5$)	Lymphocytes ($\times 10^5$)
Group 1	6.82±1.34	0.07±0.02	3.26±0.65
Group 2	12.89±2.46$^{\triangle}$	0.39±0.05$^{\triangle}$	6.54±1.57$^{\triangle}$
Group 3	19.53±4.20$^{\triangle\star}$	0.65±0.16$^{\triangle\star}$	10.56±2.36$^{\triangle\star}$

Note: Data were expressed as mean ± SD.
$^{a}P < 0.05$ versus Group 1; $^{b}P < 0.05$ versus Group 2.
Group 1: control group; Group 2: asthma group; Group 3: CVS group

Table 2. Levels of interleukin-17A, IFN-γ and TNF-α of the rats

Group	interleukin-17A		IFN-γ		TNF-α	
	BLA fluid (pg/ ml)	Plasma (pg/ml)	BLA fluid (pg/ ml)	Plasma (pg/ml)	BLA fluid (pg/ ml)	Plasma (pg/ml)
Group 1	15.91±3.26	18.52±4.98	71.34±9.96	66.16±8.09	23.43±5.67	33.45±6.75
Group 2	26.73±5.53[a]	30.04±5.30[a]	48.57±6.55[a]	40.32±5.76[a]	41.23±8.98[a]	45.06±7.80[a]
Group 3	43.85±6.25[ab]	49.88±7.96[ab]	52.43±6.64[a]	46.82±6.90[a]	46.74±9.43[a]	49.07±8.53[a]

Note: Data were expressed as mean ± SD.
[a] P < 0.05 versus Group 1; [b] P < 0.05 versus Group 2.
Group 1: control group; Group 2: asthma group; Group 3: CVS group

Ultrastructural changes of type I pneumocytes

Type I pneumocytes are important for maintaining normal lung function. We observed the ultrastructure of type I pneumocytes under the transmission electron microscope. We found no significant abnormalities of the cell from control rats. The type I pneumocyte from the rat in asthma group showed swollen mitochondria with broken cristae. When exposed to CVS, compaction, segregation and localization on the nuclear envelope of the nuclear chromatin in the type I pneumocyte were observed, theses morphologic changes indicated apoptosis of the cell. The ultrastructural change indicated that CVS induced damage to the type I pneumocyte.

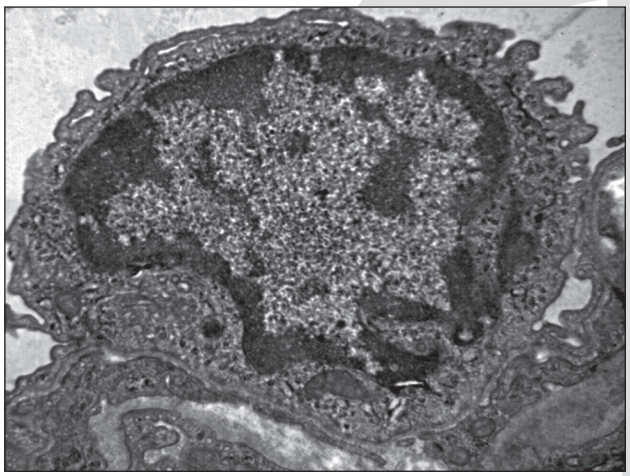

Figure 3. Type I pneumocyte from the rat in CVS group

Discussion

Psychological stress, which can lead to asthma exacerbation, occurs at high rates in asthmatic patients [28, 29]. A wealth of studies has shown that the balance between Th1 cytokines and Th2 cytokines plays a role respectively in asthma and in psychological stress. Yet, little information and only a few studies have been developed to detect changes of cytokines when the cases have both asthma and psychological disorders. Our previous study showed that chronic stress could increase levels of Th1 cytokines IL-1β and IL-6 in a rat model of asthma [1]. In the present study, we examined levels of interleukin-17, IFN-γ and TNF-α in OVA-induced asthmatic rats. The data provide evidence for the influences of CVS on Th1 and Th17 cytokines and on type I pneumocytes in asthmatic animals.

IFN-γ, a Th1 cytokine involved in asthma, was reported can inhibit Th2 cell function [30]. IFN-γ could reduce recruitment of leukocytes including lymphocytes and eosinophils, and could inhibit airways reactivity and airway obstruction in asthmatic animals, which would alleviate asthma exacerbation [31-35]. Studies showed that levels of IFN-γ were decreased in asthma [36], in contrast, some other studies showed increased levels of IFN-γ in some asthmatic patients [37, 38]. Further, IFN-γ is also involved in psychological disorders [39]. Depressed patients had decreased levels of IFN-γ [10], however, some study reported that increased level of INF-γ was observed in depressed patients [11]. A recent study by Xiang L, et al [40], showed IFN-γ was increased immediately and 1 h after acute stressor in normal volunteers. But how the levels of IFN-γ change in cases with both asthma and psychological stress has not been evaluated. In the present study, our result showed that the asthma rats had lower levels of IFN-γ in BAL fluid and in plasma as compared with the control rats, indicating the suppression of Th1 cell activity. One group of the asthmatic rats was exposed to CVS, we found no significant difference of IFN-γ levels compared to the asthmatic rats wit-

hout CVS exposure. The data indicated that CVS did not influence asthma via the IFN-γ pathway.

TNF-α, another Th1 cytokine involved in inflammation, can promote asthma exacerbation [41, 42]. Increased levels of TNF-α were found in cases with asthma [43, 44]. TNF-α is also involved in psychological disorders. Studies showed that people with depression had lower levels of TNF-α [10]. By contract, Rouhani FN found high expression of TNF-α in the brain of rat exposed to chronic mild stress (Rouhani FN, 2005). Another more recent study also showed that chronic variable stress significantly increased the levels TNF-α in the hippocampus of Wistar rats [46]. Our previous studies demonstrated that chronic stress could induce depressive-like behaviors in rats [1]. In the present study, we found TNF-α level of the rats in asthma group increased in BAL fluid and in plasma compared to the control rats. Exposed to chronic variable stress, the rats in CVS group had similar level of TNF-α with the asthma rats in asthma group. The data suggested that CVS had no significant effect on levels of Th1 cytokine TNF-α in asthma rats.

TH17 is a subset of $CD4^+$ T cells. Recently, more and more attention was focused on the role of Th17 cell in inflammation, such as asthma [14-17]. Patients with asthma had increased levels of Th17 in BAL fluid, airway tissue and blood [18, 19]. High levels of TH17 could exacerbate airway inflammation [20-22]. Th17 responses in chronic allergic airway inflammation abrogated regulatory T-cell-mediated tolerance and contribute to airway remodeling in mice [25]. IL-17A could promote human airway smooth muscle cell proliferation [24]. Studies showed that Th17 cell also play important role in pathogenesis of depression [26, 27]. A recent study by Beurel E, et al, demonstrated that learned helplessness and chronic restraint stress elevated mouse brain Th17 cells. But, how the levels of TH17A change in casess with both asthma and psychological disorders has not been evaluated. Our study showed that asthma rats had higher levels of TH17A than the control animals. When exposed to CVS, the rats had higher levels of TH17A compared to the asthmatic animals without CVS exposure. The data indicated that CVS further increased levels of TH17A in asthmatic rats.

Type I pneumocytes are important for maintaining normal lung function. In the present study, the cell from control rats showed no significant abnormalities. The type I pneumocyte from the rat in asthma group showed swollen mitochondria with broken cristae. When exposed to CVS, compaction, segregation and localization on the nuclear envelope of the nuclear chromatin in the type I pneumocyte, which indicated apoptosis of the cells, were observed under the transmission electron microscope. The morphologic changes suggest that CVS induced cellular damage to type I pneumocyte, which would decrease the lung function.

In conclusion, chronic various stress increased the level of Th17 cytokine Th17A, but had no effects on Th1 cytokines IFN-γ and TNF-α in asthma rats. Taken together with the data we reported previously [1], we conclude that chronic various stress might influence asthma via Th2 and Th17 cytokines rather than via Th1 cytokines.

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Reference

1. *Chengde Li, Shumei Mao, Yuliang Wang, Hongwei Sun. Effects of Combination Therapy of Dexamethasone and Fluoxetine on Levels of Interleukin-1β and Interleukin-6 in a Rat Model of Asthma with Depressive-like Behaviors. HealthMED. 2012, 6(6): 1998-2003.*

2. *Rod NH, Kristensen TS, Lange P, et al. Perceived stress and risk of adult-onset asthma and other atopic disorders: a longitudinal cohort study. Allergy. 2012, 67(11): 1408-1414.*

3. *Martin MA, Olson D, Mosnaim G, et al. Recruitment, asthma characteristics, and medication behaviors in Midwestern Puerto Rican youth: data from Project CURA. Ann Allergy Asthma Immunol. 2012, 109(2): 121-127.*

4. *Van Lieshout RJ, Bienenstock J, MacQueen GM. A review of candidate pathways underlying the association between asthmaand major depressive disorder. Psychosom Med. 2009, 71(2): 187-195.*

5. *Kim YH, Park CS, Lim DH, et al. Beneficial effect of anti-interleukin-33 on the murine model of allergic inflammation of the lower airway. J Asthma. 2012, 49(7): 738-743.*

6. *Nakagome K, Nagata M. Pathogenesis of airway inflammation in bronchial asthma. Auris Nasus Larynx. 2011, 38(5): 555-563.*

7. *Hong HJ, Kim E, Jung MY, et al. AIMP1 deficiency enhances airway hyperreactivity in mice via increased TH2 immune responses. Clin Immunol. 2012, 143(3): 256-265.*

8. *Ahmad T, Mabalirajan U, Hasija K, et al. Mepacrine treatment attenuates allergic airway remodelingsegregated from airway inflammation in mice. Int Immunopharmacol. 2011, 11(1): 74-78*

9. *Mitchell C, Provost K, Niu N, et al. IFN-γ acts on the airway epithelium to inhibit local and systemic pathology in allergic airway disease. J Immunol. 2011, 187(7): 3815-3820.*

10. *Song C, Halbreich U, Han C, et al. Imbalance between pro- and anti-inflammatory cytokines, and betweenTh1 and Th2 cytokines in depressed patients: the effect of electroacupuncture or fluoxetine treatment. Pharmacopsychiatry. 2009, 42(5): 182-188.*

11. *Myint AM, Leonard BE, Steinbusch HW, Kim YK. Th1, Th2, and Th3 cytokine alterations in major depression. J Affect Disord. 2005, 88(2): 167-173.*

12. *Podlipný J, Hess Z, Vrzalová J, et al. Lower Serum levels of interleukin-6 in a population sample with symptoms of depression than in a population sample without symptoms of depression. Physiol Res. 2010, 59: 121-126.*

13. *Gabbay V, Klein RG, Alonso CM, et al. Immune system dysregulation in adolescent major depressive disorder. J Affect Disord. 2009, 115(1-2): 177-1782.*

14. *Song L, Guo Y, Deng Q, Li J. TH17 functional study in severe asthma using agent based model. J Theor Biol. 2012, 309: 29-33.*

15. *Silverpil E, Lindén A. IL-17 in human asthma. Expert Rev Respir Med. 2012, 6(2): 173-186.*

16. *Besnard AG, Togbe D, Couillin I, et al. Inflammasome-IL-1-Th17 response in allergic lung inflammation. J Mol Cell Biol. 2012, 4(1): 3-10.*

17. *Wei B, Zhang H, Li L, et al. T helper 17 cells and regulatory T-cell imbalance in paediatric patients with asthma. J Int Med Res. 2011, 39(4): 1293-1305.*

18. *Al-Ramli W, Préfontaine D, Chouiali F, et al. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. J Allergy Clin Immunol. 2009, 123(5): 1185-1187.*

19. *Hashimoto T, Akiyama K, Kobayashi N, Mori A. Comparison of IL-17 production by helper T cells among atopic and nonatopic asthmatics and control subjects. Int Arch Allergy Immunol. 2005, 137 Suppl 1: 51-4.*

20. *Kolls JK, Kanaly ST, Ramsay AJ. Interleukin-17: an emerging role in lung inflammation. Am J Respir Cell Mol Biol. 2003, 28(1): 9-11.*

21. *van den Berg A, Kuiper M, Snoek M, et al. Interleukin-17 induces hyperresponsive interleukin-8 and interleukin-6 production to tumor necrosis factor-alpha in structural lung cells. Am J Respir Cell Mol Biol. 2005, 33(1): 97-104.*

22. *Wakashin H, Hirose K, Maezawa Y, et al. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. Am J Respir Crit Care Med. 2008, 178(10): 1023-1032.*

23. *Yu J, Oh MH, Park JU, et al. Epicutaneous exposure to staphylococcal superantigen enterotoxin B enhances allergic lung inflammation via an IL-17A dependent mechanism. PLoS One. 2012, 7(7): e39032.*

24. *Chang Y, Al-Alwan L, Risse PA, et al. Th17-associated cytokines promote human airway smooth muscle cell proliferation. FASEB J. 2012, 26(12): 5152-5160.*

25. *Zhao J, Lloyd CM, Noble A. Th17 responses in chronic allergic airway inflammation abrogate regulatory T-cell-mediated tolerance and contribute to airway remodeling. Mucosal Immunol. 2012 Aug 15. doi: 10.1038/mi.2012.76. [Epub ahead of print]*

26. *Hong M, Zheng J, Ding ZY, et al. Imbalance between Th17 and Treg Cells May Play an Important Role in the Development of Chronic Unpredictable Mild Stress-Induced Depression in Mice. Neuroimmunomodulation. 2012, 20(1): 39-50.*

27. *Chen Y, Jiang T, Chen P, et al. Emerging tendency towards autoimmune process in major depressive patients: a novel insight fromTh17 cells. Psychiatry Res. 2011, 188(2): 224-230.*

28. *Loerbroks A, Herr RM, Subramanian S, Bosch JA. The association of asthma and wheezing with major depressive episodes: an analysis of 245 727 women and men from 57 countries. Int J Epidemiol. 2012, 41(5): 1436-1444.*

29. *Peters TE, Fritz GK. Psychological considerations of the child with asthma. Child Adolesc Psychiatr Clin N Am. 2010, 19(2): 319-333, ix.*

30. *Gajewski TF, Fitch FW. Anti-proliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine*

helper T lymphocyte clones. J Immunol. 1988, 140: 4245–4252.

31. *Huang TJ, MacAry PA, Eynott P, et al. Allergen-specific Th1 cells counteract efferent Th2 cell-dependent bronchial hyperresponsiveness and eosinophilic inflammation partly via IFN-gamma. J Immunol. 2001, 166: 207–217.*

32. *Mitchell C, Provost K, Niu N, et al. IFN-γ acts on the airway epithelium to inhibit local and systemic pathology in allergic airway disease. J Immunol. 2011, 187(7): 3815-3820.*

33. *Kim YS, Choi SJ, Choi JP, et al. IL-12-STAT4-IFN-gamma axis is a key downstream pathway in the development of IL-13-mediated asthma phenotypes in a Th2 type asthma model. Exp Mol Med. 2010, 42(8): 533-546.*

34. *Nakanishi K, Tsutsui H, Yoshimoto T. Importance of IL-18-induced super Th1 cells for the development of allergic inflammation. Allergol Int. 2010, 59(2): 137-141.*

35. *Isogai S, Athiviraham A, Fraser RS, et al. Interferon-gamma-dependent inhibition of late allergic airway responses and eosinophilia by CD8+ gammadelta T cells. Immunology. 2007, 122(2): 230-238.*

36. *Renzi PM, Turgeon JP, Marcotte JE, et al. Reduced interferon-gamma production in infants with bronchiolitis and asthma.Am J Respir Crit Care Med. 1999, 159: 1417–1422.*

37. *Waserman S, Nair P, Snider D, et al. Local and systemic immunological parameters associated with remission of asthma symptoms inchildren. Allergy Asthma Clin Immunol. 2012, 8(1): 16.*

38. *Schmidt-Weber CB, Kunzmann S, Blaser K. TGF-beta-mediated control of allergen-specific T-cell responses. Curr Allergy Asthma Rep. 2002, 2(3): 259-262.*

39. *Litteljohn D, Cummings A, Brennan A, et al. Interferon-gamma deficiency modifies the effects of a chronic stressor in mice: Implications for psychological pathology. Brain Behav Immun. 2010, 24(3): 462-473.*

40. *Xiang L, Del Ben KS, Rehm KE, Marshall GD Jr. Effects of acute stress-induced immunomodulation on TH1/TH2 cytokine and catecholamine receptor expression in human peripheral blood cells. Neuropsychobiology. 2012, 65(1): 12-19.*

41. *Howarth PH, Babu KS, Arshad HS, et al. Tumour necrosis factor (TNFalpha) as a novel therapeutic target in symptomatic corticosteroiddependent asthma. Thorax. 2005. 60(12): 1012-8.*

42. *Berry MA, Hargadon B, Shelley M, et al. Evidence of a role of tumor necrosis factor alpha in refractory asthma. N Engl J Med 2006, 354: 697–708*

43. *Swierczyńska-Machura D, Walusiak-Skorupa J, Nowakowska-Świrta E, et al. Immunological determinants in a murine model of toluene diisocyanate-induced asthma. Int J Occup Med Environ Health. 2012 Nov 24. [Epub ahead of print]*

44. *Oettgen HC. Mast cells and tumour necrosis factor alpha (TNF-α): partners in crime in asthma pathogenesis. Clin Immunol. 2011, 140(1): 1-2.*

45. *Rouhani FN, Meitin CA, Kaler M, et al. Effect of tumor necrosis factor antagonism on allergen mediated asthmatic airway inflammation. Respir Med. 2005, 99(9): 1175-1182.*

46. *Tagliari B, Tagliari AP, Schmitz F, et al. Chronic variable stress alters inflammatory and cholinergic parameters in hippocampus of rats. Neurochem Res. 2011, 36(3): 487-493.*

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Does Yersinia infection have a role in the etiopathogenesis of Behçet's disease? A seroprevalence study

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Abstract

Objectives: The primary aim of this study is to investigate the relationship between Behçet's disease and yersinia infection in Turkey. The secondary aims are to find any correlation between antibody titer and disease duration or immunosuppressive therapy or disease activity.

Patients and Methods: Yersinia IgG, IgM and IgA antibodies were tested by quantitative micro ELISA in the sera of patients with Behçet's disease (BD) (n=101), rheumatoid arthritis (RA) patients as disease controls (n=38) and healthy controls (HC)(n=43).

Results: Serum Yersinia IgM antibody levels were significantly elevated in BD (13.6+0.93 U/ml) compared to RA (7.97+3.55 U/ml) and HC (7.34+3.11 U/ml) cases (p=0.000). The quantitative test results are grouped as positive, low titer positive and negative. Yersinia IgA, IgM, IgG seropositivity (>24 U/ml) in BD and RA were 4%, 4%, 15% and 13%, 0%, 18%, respectively. Yersinia IgA, IgM, IgG antibody positivity in HC group were 7%, 0%, 11%, respectively. Yersinia IgG and IgA antibody levels were not significantly different between groups. No correlation was found between Yersinia antibody titers and duration of disease, immunosuppressive therapy or disease activation in BD.

Conclusion: These results do not suggest a role for *Y. enterocolitica* and *Y. pseudotuberculosis* infection in the etiopathogenesis of Behçet's disease. However in the light of recent studies, the role of Yersinia infection in the etiopathogenesis of Behçet's disease should be clarified by molecular and genetic tests in future studies.

Key words: Yersinia antibodies, seroprevalence, Behçet's disease, rheumatoid arthritis.

Introduction

Hulusi Behçet gave his name to a multisystem vasculitic syndrome of unknown etiopathogenesis, that was characterized by recurrent oral ulcers, genital ulcers and hypopyon uveitis in 1937 (1). The disease occurs endemically in the geographic areas along the ancient Silk Road with the highest prevalence reported from Turkey (300 cases/100000 population) (1). A combination of genetic susceptibility, environmental, infectious and immunological factors have been postulated as causative agents (1,2).

Y. enterocolitica and *Y. pseudotuberculosis* lead to a variety of clinical manifestations such as uveitis, gastroenteritis, osteomyelitis, erythema nodosum, reactive arthritis by the help of their plasmid- and chromosome-encoded virulence proteins. In the newly discovered virulence mechanism termed type III secretion, Yop J (Yop P in *Y. enterocolitica*) is the key plasmid-encoded virulence protein in the inflammatory response to Yersinia (3-5). In the pathogenic function of Yersinia infection, Yop J/P interrupt two major cell signaling cascades: mitogen activated protein kinase and the NF-κB pathways (6). Yop P also utilises ubiquitin, a low molecular weight heat shock protein (HSP) that is involved in posttranslational modification. Upon Yersinia infection whether the induction of apoptosis results from the loss of NF-κB activation and/or loss of ubiquitin is still unknown (5). HSPs are highly conserved proteins synthesized when cells are exposed to stressful stimuli such as anoxia, infection or toxic agents and are perfect targets for autoimmune response (7). Anti-HSP immune response induced by bacterial infections may produce antibodies that cross-react with bacterial and human proteins. Many investi-

gators suggest that Yersinia HSP antibodies could trigger an autoimmune reaction through molecular mimicry between human and microbial HSPs (5-9).

BD and Yersinia infections share various clinical similarities, including mucocutaneous manifestations, uveitis and arthritis, thus raising the possibility of certain etiopathogenic factors common to both diseases. Hence, the purpose of the present investigation was to evaluate the prevalence and clinical correlations of antibodies against *Y. enterocolitica* and *Y. pseudotuberculosis* among patients with BD and compared it with that of healthy and disease controls to determine the role of this bacteria in the etiology of BD.

Methods

The study group included 101 adult outpatients with BD (44 male, 57 female, mean age 43.34 ± 8.79 years, range 21-65) who fulfilled the criteria of the International Study Group for the diagnosis of Behçet's disease (13). Patients (n=67) with any active signs or symptoms (mucocutaneous symptoms, arthralgia/artritis, headache, vertigo, eye or neurological symptoms)or any organ/vascular involvement were considered to have active disease. Thirtyeight adult RA patients (8 male, 30 female, mean age 47.39 ± 10.0 years, range 29-70) fulfilling the 1987 American College of Rheumatology criteria for the disease (14) and 43 normal healthy adult volunteers of hospital staff members (17 male, 26 female, mean age 44.11 ± 7.15 years, range 31-57) were included as controls. The groups did not differ significantly with respect to sex and age. At the time of blood withdrawal, each subjects in the groups were asked of any symptoms of acute and/or chronic enteric or urinary infections, disease duration and immunosuppressive therapy that they were taking. In the control group, no subject reported symptoms of enteric or urinary infections for the last one month. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), whole blood count, antinuclear antibody (ANA), serum immunoglobulins (IgG, IgA, IgM), antibodies (IgG, IgA, IgM) against all serotypes of *Y. enterocolitica* and *Y. pseudotuberculosis* were determined in each group.

Sera obtained from patients and controls were kept at –20^{0}C until antibodies against Yersinia were determined by commercially available indirect sandwich enzyme linked immunosorbent assay (ELISA) test kits (recomWell Yersinia IgG/IgA/IgM, Mikrogen, Germany). The test procedures were performed according to manufacturer's protocol. In the quantitative evaluation, the antibody activity levels in units per ml are assigned to the extinction values using a formula [U/ml sample = (extinction sample/extinction cutoff) x 20] given by the manufacturer. 20≤ U/ml sample ≤ 24 were accepted borderline, those above 24 U/ml being positive and those below 20 U/ml being negative. The manufacturer suggested that cross reactivity with Brucella and any interference with RF and Ebstein-Barr virus IgM do not occur.

The study protocol was approved by the Ethical and Research Committee of Ankara University and the participants voluntarily agreed to participate to that study before collecting blood samples with a written informed consent. The study was performed in accordance with the Declaration of Helsinki and participant anonymity has been preserved.

Statistical Analysis

Statistical analysis was performed on PC using SPSS for Windows, version 12.0. Statistical evaluation was carried out by an ANOVA test on the transformed data and was followed by a pairwise *post hoc* comparison using the least significant difference (LSD) method to compare each group. Pearson's rank correlation test, independent sample T test and Mann-Whitney U test were used in the correlation analysis. A p value ≤0.05 was considered significant.

Results

The demographic and clinical features of the patient and control groups are shown in Table 1. The disease duration (range 1-34) was older than fifteen years in thirtyfour BD patients. When patients with disease duration older or lesser than 15 years were compared, no significant differences were observed in terms of each Yersinia antibody titers (p>0.05). Upon there was no correlation between acute infection and any Yersinia antibody titers or serum immunoglobulin levels or WBC; significant differences between chronic infection and Yersinia IgA titers or serum IgA were found (p<0.05). Colchicine alone were taken by 80% of BD patients and

Table 1. The demographic and clinical features of the patient and control groups

		Behçet's disease (BD) (n=101)	Rheumatoid arthritis (RA) (n=38)	Healthy controls (HC) (n=43)
Male/female		44/57	8/30	17/26
Mean age(year)		43.34 ± 8.79	47.39 ± 10	44.11 ± 7.15
Mean disease duration (year)		10.97	8.46	-
ANA positivity		3	11	1
RF positivity		3	33	4
Enteric/urinary infection	Acute	20	0	0
	Chronic	40	0	0

10% of patients were treated with both colchicine and any immunosuppressive (azathioprine, cyclosporine, steroids, cyclophosphamide, methotrexate, anti- tumor necrosis factor, leflunomide, hydrochloroquin) therapy. In BD patients, no significant differences between immunosuppressive therapy used and antibodies to *Y. enterocolitica* and *Y. pseudotuberculosis* in neither Ig class. But, Mann- Whitney U test revealed a significant difference in BD patients between immunosuppresive therapy used and serum IgG levels or WBC.

The clinical symptoms observed in BD patient group at the time of blood withdrawal are shown in Table 2. Thirtyfour (33.6%) BD patients had neither active symptom nor any organ involvement. Deep vein thrombosis was observed in 8 BD patients. Four patients had gastrointestinal involvement thought to be due to BD. One patient had both neurological and gastrointestinal involvement of BD. Patients (n=67) with any active signs or symptoms or any organ/ vascular involvement due to BD were considered to have active disease. No correlation between active disease and anti-Yersinia antibodies or serum immunglobulins or WBC (p>0.05).

Table 2. Clinical symptoms of patients with Behçet's disease (n=101)

Symptom	n (%)
Oral ulcer	43 (42.6)
Genital ulcer	2 (2)
Erythema nodosum	12 (11.9)
Arthritis/arthralgia	22 (21.8)
Neurological involvement	7 (6.9)
Eye manifestations	9 (8.9)
Active symptom/sign*	58 (57.4)

**any active signs or symptoms observed at the time of blood withdrawal.*

Serum IgA levels were significantly lower ($p=0.02$) in HC than in BD or RA. CRP levels were significantly higher in RA than other groups. The quantitative Yersinia IgM antibody levels were found significantly higher in BD compared to RA or HC cases. Serum ESR levels were comparable in the three groups (Table 3).

Yersinia IgA seropositivity (>24U/L) was detected by ELISA in 12 subjects in three groups. The distribution of these were as: four patients (4%) with BD, five (13.1%) with RA, and three (7%)

Table 3. CRP, ESR, Serum IgG/IgA/IgM, Yersinia IgG/IgA/IgM, WBC results of three groups

Results (mean±SD,SE)*	BD patients	RA controls	HC	p
CRP mg/L	6.68 + 1.02	19.88 + 3.82	3.75 + 1.51	0.000
ESR mm/hr	16.5 + 1.71	30.31 + 4.54	8.32 + 0.96	0.000
Serum IgG g/L	12.36 - 2.72	12.99 - 3.83	11.53 - 1.59	0.062
Serum IgA g/L	2.47 + 0.12	2.69 - 1.22	1.84 - 0.61	0.002
Serum IgM g/L	1.37 + 0.07	1.24 + 0.1	1.16 + 0.09	0.240
Y. IgG U/ml	20.38 + 2.34	16.61 + 2.78	15.78 + 4.39	0.489
Y. IgA U/ml	12.03 + 0.62	15.29 + 2.6	11.09 + 1.46	0.128
Y. IgM U/ml	13.6 + 0.93	7.97 - 3.55	7.34 - 3.11	0.000
WBC	7384 - 2443	7757 - 2354	7088 - 2044	0.438

**SD: standard deviation; SE: standard error; $p \leq 0.05$ significant*

Table 4. The comparison of the patient and control groups for Y. enterocolitica and Y. pseudotuberculosis seropositivity.

Yersinia antibodies	BD patients (%) n=101		RA controls (%) n=38		HC (%) n=43	
	borderline	positive	borderline	positive	borderline	positive
IgA	1 (1)	4 (4)[a]	1 (2.6)	5(13.1)[a]	1(2.3)	3(7)[b]
IgM	2 (2)	4 (4)	0 (0)	0(0)	0 (0)	0(0)
IgG	8 (8)	15(15)[a]	1 (2.6)	7(18.4)[a]	0 (0)	5(11.6)[b]

[a] *Three patients IgG and IgA positive*
[b] *Two healthy staff member IgG and IgA positive*

with HC. Both Yersinia IgA and IgG antibody seropositivities were detected in three BD patients, three RA controls and two HC group. Yersinia IgM seroreactivity was not detected in two control groups and all Yersinia IgM seropositivities in BD were isolated seropositivities. The comparison of the groups according to Yersinia seropositivity (positive and borderline) is shown in Table 4. Borderline results were not confirmed by additional tests.

Discussion

The incidence of yersiniosis was reported as 8.7/100000 in Germany, 14.1/100000 in Finland, 6.5/100000 in Sweden, 2.7/100000 in Norway and 5.3/100000 in Denmark (12). Yersinia is declared to be one of the diseases included in the notification system of communicable diseases in Turkey since 2007 which will lead to collect the important epidemiological datas in our health system (13). The incidence of yersiniosis will remain mysterious until that time. Our study contributes new datas about the seroprevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* not only in BD but also in RA patients and in healthy Turkish population. The percentage of any Ig class antibodies to Yersinia in 28 BD patients was 32% and 20% in control population consisting of both diseased and healthy subjects in Turkey in 1987 (14). After this pilot study, the study group was expanded to include 100 BD patients, 48 diseased control population and 49 healthy Turkish individuals in which one third of both the BD and control group with other diseases (30% and 29%, respectively) had increased levels of antibodies against any Yersinia serotype in 1989 (15). Gedikoğlu et al reported 13.26% of Yersinia seropositivity in 392 healthy Turkish individuals in the province of Bursa in 1990 (16). The Yersinia seropositivity of IgM, IgG and IgA was reported as 22%, 30%, 22% respectively in patients with reactive arthritis and as 12.5%, 12.5% and 0% in healthy individuals in the study of Etiz et al (17). We found Yersinia seropositivity of IgM, IgG and IgA as 4%, 15%, 4% in BD group; as 0%, 18%, 13% in RA group and as 0%, 12%, 7% in healthy group. The IgM seropositivity against Yersinia in BD was an isolated positivity; neither IgG nor IgA seropositivity was detected in those patients. Although the study groups of two studies were not exactly similar to compare, we found no Yersinia IgM seropositivity and lesser percentage of IgG seropositivity in healthy individuals in respect to the study of Etiz et al (17).

The precise etiology of BD is still unknown today. There were studies which investigated the role of infectious agents in the etiopathogenesis of BD in the literature. Viral and bacterial agents like hepatitis G virus, hepatitis B virus, Parvovirus B19, herpes simplex virus-1, Epstein-Barr virus, cytomegalovirus, human herpes virus 8, *Streptococcus* spp., *Chlamydia* spp., *Borrelia burgdorferi*, *Campylobacter* spp., *Chlamydia* spp., *Coxiella* spp., *Listeria* spp., *Leptospira* spp., *Salmonella* spp., *Mycobacterium paratuberculosis* had all been studied and there were different suggestions about their association with BD (14, 15, 18-20).

Heat shock proteins (HSP) are the second important headline trying to explain the infectious etiopathogenesis of BD. The high homology between human and microbial HSP in addition to their physiological roles were shown by the previous studies in which HSP had the ability to modulate autoimmune diseases, atherosclerosis, inflammation and tumor cell immunogenicity (7-9, 21). HSP 60 kDa of *Y. enterocolitica* was found to be involved in the etiology of uveitis in BD (8-9). The interaction between the pathogen and the host immune system is described mostly in

molecular level in the last decade. There is a very well known mechanism called type III secretion system of Yersinia which blocks cell function and its communication system by injecting Yersinia outer proteins (Yops) (3, 4, 6).

The clinical signs and symptoms of BD with ocular, mucocutaneous and joint involvement resembles seronegative spondyloarthropathies especially postinfectious developing reactive arthritis. Yersiniosis is one of the infectious diseases causing reactive arthritis. The diagnosis of *Yersinia* spp. depends mostly on the serological tests in the postinfectious period of reactive arthritis (17,22).

In the light of the studies reported in the literature about HSP, Yersinia and BD; our aim was to investigate causative relationship between Yersinia and BD serologically after a similar study published in 1989 from Turkey. Our study group was larger than most of the previous studies reported from Turkey (14,15). The limitation of our study was the diagnostic method for Yersinia which depended on only serological tests as culture technique does not recommended in the identification of postinfectious pathogen in reactive arthritis. But borderline seropositivities were not confirmed by a second ELISA test. Molecular and genetic studies about yersiniosis are popular in the last decade. Yersinia virulence factor, YopJ acting as an ubiquitin degrading proteolyse was shown to be resposible from inhibition of NF-κB and MAPK signaling (23). Ubiquitin has important functions in the immune system and Yops are the first bacterial member of ubiquitin-like protein proteases that lead SUMO attack (5,6). Small ubiquitin-like modifier 4 (SUMO4), located on 6q25 has been found to be involved in autoimmune and inflammatory responses (24). SUMO4 gene polymorphisms in Chinese Han patients and in Tunisian population with BD were reported in the literature and the authors suggested that SUMO4 gene may be a general autoimmunity gene (24-26).

We found statistically significant differences between chronic infection and Yersinia IgA titers or serum IgA as in accordance with the study reported by Etiz et al (17). Although any one of the Ig class of Yersinia was not found to be associated with acute infectious symptoms in BD, Yersinia IgA titer may be accepted as a prognostic marker in the evaluation of disease chronicity in autoimmune rheumatic diseases. Neither isolated Yersinia IgA seropositivity nor the combined Yersinia IgG and IgA seropositivities that was found in the healthy control group gave us an impression to follow up this group in terms of developing infectious sequela secondary to yersiniosis in our mind.

Conclusion

Our serology-based results did not find any correlation between yersiniosis and BD to make a clear statement in the etiopathogenesis. Our study is unique to add new Yersinia seroprevalance study in BD from Turkey after the year 1989. We think molecular and genetic tests such as SUMO4 gene polymorphisms, investigations of anti-ubiquitin antibodies in BD at large samples will result better scientific outcomes in the etiopathogenesis of BD.

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References

1. *Fessler BJ. Behçet's syndrome. In: Koopman WJ, Moreland LW, editors. Arthritis and Allied Conditions. A Textbook of Rheumatology. Philadelphia: Lippincott Williams & Wilkins, 2005: 1835-44.*

2. *Ayaşlioğlu E, Düzgün N, Erkek E, İnal A. Evidence of chronic Chlamydia pneumoniae infection in patients with Behçet's disease. Scand J Infect Dis 2004; 36: 428-30.*

3. *Cornelis GR. Yersinia type III secretion: send in the effectors. J Cell Biol 2002; 158: 401-8.*

4. *Ellison DW, Young B, Nelson K, Miller VL. YmoA negatively regulates expression of invasin from Yersinia enterocolitica. J Bacteriol 2003; 185: 7153-9.*

5. *Hrycaj P, Lacki JK. Antibodies to ubiquitin in relation to Yersinia infection status in patients with ankylosing spondylitis. Clin Rheumatol 2003; 22: 33-6.*

6. *Cornelis GR, Denecker G. Yersinia lead SUMO attack. Nature Med 2001; 7: 21-3.*

7. *Direskeneli H, Ekşioğlu-Demiralp E, Yavuz Ş, Ergün T, Shinnick T, Lehner T, et al. T cell responses to 60/65 kDa heat shock protein derived peptides in Turkish patients with Behçet's disease. J Rheumatol 2000; 27: 708-13.*

8. *Tanaka T, Yamakawa N, Yamaguchi H, Okada AA, Konoeda Y, Ogawa T, et al. Common antigenicity between Yersinia enterocolitica-derived heat-shock protein and the retina, and its role in uveitis. Ophthalmic Res 1996; 28: 284-8.*

9. *Cancino-Diaz JC, Vargas-Rodriguez L, Grinberg-Zylberbaum N, Reyes-Lopez MA, Dominguez-Lopez ML, Pablo-Velazquez A, et al. High levels of IgG class antibodies to recombinant HSP60 kDa of Yersinia enterocolitica in sera of patients with uveitis. Br J Ophthalmol 2004; 88: 247-50.*

10. *International Study Group for Behçet's Disease. Criteria for diagnosis of Behçet's disease. Lancet 1990; 335: 1078-80.*

11. *Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31: 315-24.*

12. *Fredriksson-Ahomaa M, Korkeala H. Molecular epidemiology of Yersinia enterocolitica 4/O: 3. In: Skurnik M, Bengoechea JA, Granfors K, eds. The Genus Yersinia. Entering the Functional Genomic Era. New York: Kluwer Academic /Plenum Publishers, 2003: 295-302.*

13. *Bulaşici Hastaliklar Sürveyans ve Kontrol Esaslari Yönetmeliği. 30.05.2007 tarih ve 26537 sayili Resmi Gazete. www.resmigazete.gov.tr [accessed on 25 September 2012]*

14. *Tovianen A, Lahesmaa-Rantala R, Meurman O, Sappinen O, Kosunen T, Yazici H, et al. Antibodies against Yersinia, Campylobacter, Salmonella and Chlamydia in patients with Behçet's disease. Arthritis Rheum 1987; 30: 1315-7.*

15. *Lahesmaa-Rantala R, Tovianen A, Granfors K, Yurdakul S, Hamuryudan V, Yazici P. Anti-Yersinia antibodies in patients with Behçet's disease. Arthritis Rheum 1989; 32: 1494-5.*

16. *Gedikoğlu S, Göral G, Helvaci S, Mistik R. A serological study with Yersinia enterocolitica. Mikrobiyol Bul 1990; 24: 214-7.*

17. *Etiz N, Özsan M, Cengiz AT. Reaktif artritli hastalarda Yersinia enterocolitica O: 3 antijenlerine karşi antikorlarin iki farkli yöntemle araştirilmasi. Mikrobiyol Bul 1998; 32: 201-8.*

18. *Irschick EU, Philipp S, Shahram F, Schirmer M, Sedigh M, Ziaee N, et al. Investigation of bacterial and viral agents and immune status in Behcet's disease patients from Iran. Int J Rheum Dis 2011; 14: 298-310.*

19. *Onen F, Tuncer D, Akar S, Birlik M, Akkoc N. Seroprevalence of Borrelia burgdorferi in patients with Behçet's disease. Rheumatol Int 2003; 23: 289-93.*

20. *Iannone F, Lapadula G, De Bari C, Covelli M, Pipitone V. Anti-bacterial antibodies in Behçet's disease. Clin Exp Rheumatol 1997; 15: 451-2.*

21. *Wells AD, Malkovsky M. Heat shock proteins, tumor immunogenicity and antigen presentations: an integrated view. Immunol Today 2000; 21: 129-32.*

22. *Khan MA, Sieper J. Reactive Arthritis. In: Koopman WJ, Moreland LW, eds. Arthritis and Allied Conditions. A Textbook of Rheumatology. Philadelphia: Lippincott Williams & Wilkins, 2005: 1335-55.*

23. *Zhou H, Monack DM, Kayagaki N, Wertz I, Yin J, Wolf B, et al. Yersinia virulence factor YopJ acts as a deubiquitinase to inhibit NF-kappa B activation. J Exp Med 2005 21; 202: 1327-32.*

24. *Tsurumaru M, Kawasaki E, Ida H, Migita K, Moriuchi A, Fukushima K, et al. Evidence for the role of small ubiquitin-like modifier 4 as a general autoimmunity locus in the Japanese population. J Clin Endocrinol Metab 2006; 91: 3138-43.*

25. *Hou S, Yang P, Du L, Zhou H, Lin X, Liu X, et al. SUMO+ gene polymorphisms in Chinese Han patients with Behcet's disease. Clin Immunol 2008; 129: 170-5.*

26. *Kamoun M, Ben Dhifallah I, Karray E, Zakraoui L, Hamzaoui K. Association of small ubiquitin-like modifier 4 (SUMO4) polymorphisms in a Tunisian population with Behçet's disease. Clin Exp Rheumatol. 2010; 28 (Suppl 60): S45-9.*

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Molecular frequency of enterotoxigenic Staphylococcus aureus among different isolated of food products

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Abstract

Background: *S. aureus* is a major causative agent of food poisoning. Staphylococcal food poisoning is due to the production of staphylococcal enterotoxins (*SEs*) by *S. aureus* strains contaminating foodstuffs. The aim of present study was to investigate the presence of classical enterotoxin genes in food isolates of *S. aureus* in north of Iran.

Method: For this purpose, 540 samples from different food products were collected during 6 months and tested for *S. aureus* isolation by routine bacteriological methods. The isolated bacteria were evaluated by PCR methods for detection of *sea-see*, subsequently.

Results: The results indicated that 15.7% of food samples were contaminated by *S. aureus.* Out of 85 isolates studied, 19 isolate (22.3%) were positive for at least 1 *SE* gene. Of these 9(47.3 %) were positive for *sea*, 6(31.2 %) for *sec*, 4(21%) for *sed*, 3(15.6%) for *see*, 2(10.5%) for *sea*, *sec* and 1(5.2 %) for *sea*, *sed*. No amplification corresponding to *seb* was obtained. The Number of staphylococcal enterotoxin genes in each dairy and meat group were similar.

Conclusion: This report reveals that *sea* was the most common enterotoxin gene in food isolates of *S. aureus*. These findings emphasize the need to prevent the presence of *S. aureus* strains and *SEs* production in food.

Key words: *S. aureus*, enterotoxin, food, PCR.

Introduction

Staphylococcus aureus is one of the major human pathogens which can cause various infections ranging from superficial lesions to life-threatening septicaemia. *Staphylococcus aureus* Food Poisoning (FP) is a common cause of food-borne disease worldwide. Staphylococcal food poisoning is due to the production of staphylococcal enterotoxins (*SEs*) by *S. aureus* strains contaminating foodstuffs (1). Indeed not all staphylococci produce *SE* and *SE* production maybe insufficient for contamination of food products (2).

These *SE* proteins have a remarkable ability to resist heat and acid. There for, they may not be completely denatureted by mild cooking of contaminated food. They can induce nausea in the central nervous system by stimulating neuron receptors in intestine since *SEs* are resistant to inactivation by gastrointestinal proteases (3, 4).

More than 20 various types of enterotoxins with nearly similar amino acid frequencies have been reported. Traditionally, 7 classic antigenic *SE* types have been recognized: *SEA*, *SEB*, SEC_1, SEC_2, SEC_3, *SED* and *SEE* (1, 5).

In enterotoxins, *SEA* was frequently identified in contaminated foods followed by *SEB* toxins. In United States *SEA* is the most common enterotoxin recovered from food-poisoning outbreak (77.8%) but followed by *SED* (37.5%) while *SEB* is responsible for 10% of food contamination (5, 6).

Genes of *sea* and *see* are carried by temperate bacteriophages. In the meanwhile, *SEB* and *SEC* have placed on chromosomes and *SED* is carried by plasmid (7). In addition to the classical *SEs*, new *SEs* have recently been discovered (*SEF-SEU*). But of these new *SEs* in food poisoning has not yet been clarified (7, 8).

Immunologic methods including ELISA, agglutination latex, radio immunoassay and immuno-

diffusion were widely used for detecting enterotoxins in sera. But these methods are based on gene expression, so they cannot be of help when the amount of toxin production lessens (9).

Therefore developing a rapid, sensitive method seems necessary for detecting toxins within the shortest possible time. In comparison to the very immunologic methods, PCR is preferable considering its higher sensitivity and other features (10, 11).

The aim of the present study was to examine the prevalence of 5 classic enterotoxins genes (*sea - see*) in food isolated of *S. aureus*.

Materials and Methods

Bacterial strains and media

From April 2011 to October 2011 about 540 different food products from the north of Iran were collected for analyses of the presence of *S. aureus*. Food samples were included meat products (raw meat, poultry, hamburger, hot dog, sausage) and dairy products (pastry cream, ice cream, raw milk, cheese, butter).

Initially, a 1: 10 dilution was prepared with 5g of each food sample in 45 ml Trypticase Soy Broth (TSB, Merck) at pH: 7 with the addition of 10% sodium chloride (NaCl, Merck). The homogenized samples was incubated for 18-24 h at 37°c for enrichment, then spread onto Baird-Parker Agar (BPA, Merck) supplemented with egg yolk tellurite enrichment suspension (Merck) and incubated at 37°c for 48h. The suspected colonies (black colony, convex and surrounded by clear zone and on opaque zone may appear inside the halo) were selected. *S. aureus* was confirmed by colonial morphology, Gram staining, catalase activity, mannitol fermentation and coaglutination of citrated rabbit plasma (12, 13).

The pure culture of presumptive staphylococci were sub cultured and stored in Nutrient Agar (NA, Merck) slant and maintained at -20°c, until use.

DNA Isolation

Total genomic DNA was obtained from *S. aureus* by the modified phenol chloroform extraction method. The stored bacterial strains were first sub cultured on Nutrient Broth (NB, Merck) at 37°c for 18-24 h, then 1 ml of these were centrifuged at 14000g for 2min. Then 300 µlit TE_{1x} buffer (10 mmol/l Tris-HCl, pH 8.0, 1 mmol/1 EDTA) and 10µl lysozyme (100mg/ml, sigma) were added to cells and the mixture was incubated at 37°c for 45min. Followed by the addition of 300µl sarcozyle 2% after incubation at 37°c for 30min, 30µl proteinase K (10 mg/ml, sigma) and 5µl RNase A (10mg/ml, GeNet Bio) were added and the mixture was incubated at 37°c for 1h. To this lysate 750µl of phenol chloroform (pH: 7) was added followed by centrifugation the mixture at 9000g for 10min. The supernatant was again extracted with equal volume of phenol-chloroform followed by equal volume chloroform again (9000g for 10min). DNA in the supernatant was precipitated by mixing with 2 volumes of 95% ethanol, and starting storing at -70°c for 1h. The DNA pellet was then washed with ice-cold 70% ethanol followed by centrifugation (14000g 10min). The DNA pellet was then dried at 45°C. Before use, it was suspended in sterile TE_{1x} Buffer and stored at 4°C (6, 14, 15).

PCR amplification

Primers for the detection of the genes *sea-see* (Cinnagen Co., I.R. Iran) were selected from the published sequences (Table 1) and designed to be specific of each *SE* type gene and to have similar melting temperatures (Tm) (16).

The PCR was performed in a 25µl reaction mixture containing 1µl template DNA, 2.5µl 10X PCR-buffer (50mM KCl, 1mg ml^{-1} gelatin, 10mM Tris-HCl, pH: 8.3, GeNet Bio) 3µl $MgCl_2$ (25 mM GeNet Bio), 1µl of dNTP (2.5mM GeNet Bio), 1µl of each primer (10 PM), 0.5 µl of Taq polymerase (1 ul^{-1}, GeNet Bio) and 15 µl dd H_2O. DNA amplification was carried out in a primus 96 advanced thermocycler (PEQLAB Biotechnologie GmbH, Erlangen, Germany) with the following thermal cycling: on initial denaturation at 94°C for 5min was followed by 35 cycles of amplification (denaturation at 94°C for 1min, anneling at 55°C for 1min except for *sea*, *sec*: 50°C, 59°C, and extension at 72°C for 1min), ending with a final extension at 72°C for 5min. The amplified products were shown by electrophoresis on 1.5% gel agarose. Electrophoresis was performed for 1h at 100V, while staining the amplicons with ethidium bromide (Merck). The result was visualized under UV light in gel-Doc system. The presence

Table 1. Details of primers, PCR condition and PCR product size (13, 15)

Gene	Primer	Size	Oligonucleotide sequence (5 →3')	Tm	PCR condition	PCR product size (bp)
sea	*Sea*-F	19	F 5'- TTGGAAACGGTTAAAACGA-3'	61.8	94 50 1 min 72	120
	Sea-R	19	R 5'- GAACCTTCCCATCAAAAAC-3'	61.3		
seb	*Seb*-F	19	F 5'- TCGCATCAAACTGACAAAC-3'	64.8	94 55 1 min 72	478
	Seb-R	19	R 5'- GCAGGTACTCTATAAGTGC-3'	55.5		
sec	*Sec*-F	22	F 5'- GGAGGAATAACAAAACATGAAG-3	62.2	94 59 1 min 72	458
	Sec-R	19	R 5'-AAAGGCAAGCACCGAAGTA-3'	63		
sed	*Sed*-F	20	F 5'-TTGTACATATGGAGGTGTCA-3'	57.3	94 55 1 min 72	362
	Sed-R	20	R 5'-TATGAAGGTGCTCTGTGGAT-3	59.7		
see	*See*-F	19	F 5'-TGGTAGCGAGAAAAGCGAA-3	64.3	94 55 1 min 72	494
	See-R	21	R 5'-TGTAAATAATGCCTTGCCTGA-3	63		

Table 2. Prevalence of S. aureus and SE genes in food isolates

Samples		Total	*S.aureus* Positive (%)	CI for mean 95%	Enterotoxin	sea	sec	sed	see	sea & sec	sea & sed	Personal correlation
Dairy Products	Ice cream	70	14(20%)	1.70-1.90	4(28.6%)	2	2	1	0	1	0	0.476
	Pasty cream	80	13(16.2%)	1.71-1.89	4(30.8%)	2	1	0	0	0	0	0.454
	Row milk	50	5(10%)	1.81-1.99	1(20%)	1	0	0	0	1	0	0.429
	Cheese	30	4(13.3%)	1.74-2.00	0(0%)	0	0	0	0	0	0	0.00
	Butter	40	5(12.5%)	1.77-1.98	2(40%)	0	0	1	1	0	0	0.540
Total		270	41		11	5	3	2	1	2	0	
Meat Products	Row meat	44	12(27.3%)	1.59-1.86	3(25%)	1	1	1	1	0	1	0.425
	Poultry	46	8(17.4%)	1.71-1.94	2(25%)	0	1	0	1	0	0	0.414
	Hamburger	60	15(25%)	1.60-1.83	5(33.3%)	2	0	2	1	0	0	0.432
	Hot Dog	60	6(10%)	1.80-1.97	1(14.3%)	1	0	0	0	0	0	0.358
	Sausage	60	3(5%)	1.89-2.01	0(0%)	0	0	0	0	0	0	0.00
Total		270	44		11	4	2	3	3	0	1	

of a band at the expected product size was considered a positive result (14, 17, 18).

Statistical analysis

Independent sample T test (SPSS ver. 16) was used for the statistical analysis of the gathered data. P Values below 0.05 were considered statistically significant.

Results

In the present study, 85 *S. aureus* were isolated from 540 food sample (41 isolates of the dairy products and 44 isolates of the meat products). All of 85 strains were confirmed with Gram staining and complementary biochemical tests. Then they examined for presence of enterotoxin gene by PCR method (figure 1).

Results of amplification with enterotoxin specific primers are shown in table 2.

19 (22.3%) out of 85 isolates were positive for an enterotoxin gene. Of these 9 (47.3%) were positive for *sea*, 6(31.2%) for *sec*, 4(21%) for *sed*, 3 (15.6%) for *see*, 2(10.5%) for *sea*, *sec* and 1(5.2%) for *sea*, *sed*. No amplification product corresponding to *seb* was obtained. The number of *se* positive cases in each dairy and meat products were similar (12.9%).

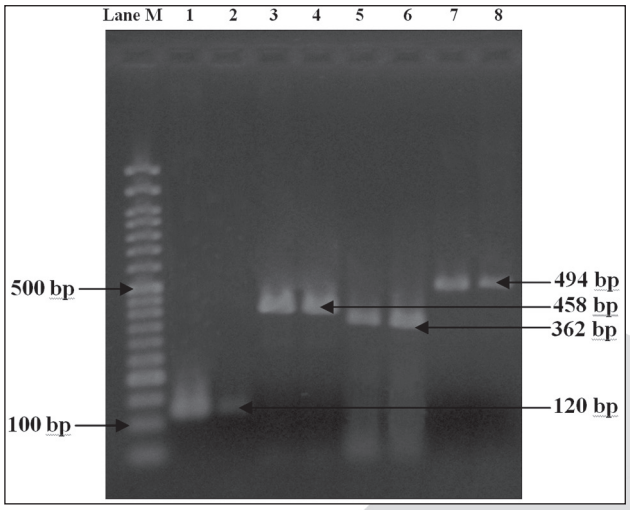

Figure 1. Agarose gel electrophoresis of the amplified PCR fragments for the Staphylococcal toxin genes. Lane M, DNA Molecular size marker (50 bpladder; Fermentas Gene Ruler). Lane 1 and 2, SEA positive isolates (120 bp). Lane 3 and 4, SEC positive isolates (459 bp). Lane 5 and 6, SED positive isolates (362 bp). Lane 7 and 8, SEE positive isolates (494 bp).

Discussion

One of the most important problems is that *S. aureus* can be responsible for food poisoning by enterotoxin production. In current study, 15.7% of all food products were contaminated by *S.aureus*. Obviously, the quantity of *S. aureus* in food products are related to many factors: the number of contaminated carriers and personnel in preparing the food, ignoring the rules of hygiene in food factories, transport systems and rate of animal contamination. All of the factors need to be controlled separately (19). Our results showed that 22.3% of isolates have one or more enterotoxin genes. Among them the *sea* genotype was the most frequent (47%) and *sec* the second more frequent (33%), which is in accordinance with the results obtained by Jay (2000), who reported that *sea* was most frequently involved in food poisoning. But this result differed from Suppajariyawat (2009), because they reported that *seb* was most frequently involved in food poisoning (20).

Pinto *et al.* (2005) revealed that 30 of food isolates positive for *se* genes and *sec* genes was most frequent (20%) and *sea* the second more frequent (13%) (13).

Another study was showed that 69% of the isolates had one or more *se* genes and the most commonly was *sea* (25%). One other hands *sea* and *seb* were responsible for 80% of collective nutritional intoxication cases in France (21).

But in the US, the *seb* is responsible for 10% of food contamination (22).

Also, in the present study, the number of *se* positive cases in each dairy and meat products was similar, respectively. The statistical analysis of current data indicated that the percentage of *S. aureus* carring *se* not depends on the source of the food (P 0.05). In other wise, the isolates collected from dairy products showed a higher incidence of *sec* genes. In contrast, presence of *sed* and *see* was significantly associated with meat products.

Considering the findings of the present study and comparing them to other studies, it can be stated that the food type and food processing method was effective on the prevalence rate of the *se* genes in *S. aureus* isolates, the distribution of the isolates containing this gene might be variable in different food products (23).

However, we should consider that detection of enterotoxin genes is not always concurrent with the toxin production. This maybe due to lower level of toxin production or mutation in regulatory regions. Thus, demonstration of toxic level for these strains is needed (4, 23). In conclusion, these findings highlight the high potential risk for consumers in the absence of strict hygienic and preventative measures to avoid the presence of S. *aureus* isolates and *SEs* production in foods, emphasizing the need for improved hygiene practices during food processing and also during the distribution and consumption of the final food products.

References

1. *Le Loir Y, Baron F, Gautier M . Staphylococcus aureus and food poisoning. Genetics and Molecular Research 2003; 2: 63-76.*

2. *Villard L, Lamprell H, Borges E, Maurin F, Noel Y. Enterotoxin D producing strains of Staphylococcus aureus are typeable by pulsed-field gel electrophoresis (PFGE). Food Microbiology 2005; 22: 261-265.*

3. *Fujikawa H, Morozumi S. Modeling Staphylococcus aureus growth and enterotoxin production in milk. Food Microbiology 2006; 23: 260-267.*

4. *Schelin J, Cohn N, Lindqvist R, Barker G, Radstrom P. The formation of Staphylococcus aureus enterotoxin in food environments and advances in risk assessment. Virulance 2011; 2(6): 580-592.*

5. *Dings M, Orwin P, Schelievert P. Exotoxins of Staphylococcus aureus. Clinical Microbiology Reviews 2000; 13(1): 16-34*

6. *Chen TR, Hsiao MH, Chiou CS, Tsen HT. Development and use of PCR primers for the investigation of C_1, C_2 and C_3 enterotoxin types of Staphylococcus aureus strains isolated from food-borne out breaks. International Journal of Food Microbiology 2001; 21: 63-70.*

7. *Pinchuk IV, Beswick EJ, Reyes VE. Staphylococcal Entrotoxins. Toxins 2010; 2: 2177-2197.*

8. *Pereira V, Lopes C, Castro A, Silva J, Gibbs P, Teixeira P. Characterization for enterotoxin production, virulence factors and antibiotic susceptibility of Staphylococcus aureus isolates from various foods in Portugal. Food Microbiology 2009; 26: 278–282.*

9. *Rahimi E, Momtaz H, Shakerian A, Kavyani HR (2011). The detection of classical enterotoxin of Staphylococcus aureus in raw com milk using the ELISA method. Turkish Journal of Veterinary and Animal Sciences 2011; 35(1).*

10. *Pourmand MR, Memarian M, Hosseini M, Bagherzadeh Yazdchi S. High prevalence of SEA gene among clinical isolateds of Staphylococcus aureus in Tehran. Acta Medical Iranica 2009; 47(5): 357-367.*

11. *Vasconcelos NG, Delourdes M. Staphylococcal Entrotoxins: molecular aspects and detection methods. Journal of public health and Epidemiology 2010; 2(3): 29-42.*

12. *Baron EJ, Fine Gold SM. Diagnostic microbiology 1990; pp 861-880.*

13. *Pinto B, Chenoll E, Azna, R. Identification and typing of food-borne Staphylococcus aureus by PCR-based techniques. Systematic and Applied Microbiology 2005; 28: 340-352*

14. *Chen TR, Chiou CSH, Tsen HY. Use of novel PCR primers specific to the genes of staphylococcal Entrotoxin G, H, I for the survey of Staphylococcus aureus strains isolated from food-poisoning cases and food sample in Taiwan. International Journal of Food Microbiology 2004; 92: 189-197.*

15. *Chiang YC, Liao WW, Fan CM, Pai WY, Chiou CS, Tsen HY (2008). PCR detection of staphylococcal Entrotoxin (SEs) N, O, Q, R, U, and survey of SE types in S. aureus isolates from food-poisoning cases in Taiwan. International Journal of Food Microbiology 2008; 121: 66-73.*

16. *Ataee RA, Karami A, Izadi M, Aghania A, Ataee MH. Molecular screening of Staphylococcal Entrotoxin b gene in clinical Islates. Cell Journal 2011; 13(3): 187-192.*

17. *Oliveria L, Rodrigues AC, Hulland C, Ruegg PL. Enterotoxin production, enterotoxin gene distribution, and genetic diversity of Staphylococcus aureus recovered from milk of cows with subclinical mastitis. AJVR 2011; 72(10): 1361-1368.*

18. *Zschock M, Bottzler D, Blocher S, Sommer hauser J, Hamann HP. Detection of genes for enterotoxin (ent) and toxic shock syndrome toxin-1(tst) in mammary isolates of Staphylococcus aureus by polymerase-chain-reaction. International Dairy Journal 2000; 10: 569-574*

19. *Imani Fooladi AA, Tavakoli HR, Naderi A. Detection of enterotoxigenic Staphylococcus aureus isolates in domestic dairy products. Iranian Journal of Microbiology 2010; 2(3): 135.140.*

20. *Suppajariyawat P, Lawharinit O, Sunthornandh P. Detection of enterotoxin-producing and methicillin- and vancomycin-resistant Staphylococcus aureus in Ready-toeat foods at Kasetsart university Bangkhen campus and kaset intersection market. Kasetsart Journal: Natural Science 2009; 43: 313-398.*

21. *Rosec JP, Gigaud O. Staphylococcal entrotoxin genes of classical and new types detected by PCR in France. International Journal of Food Microbiology 2002; 72: 61-70.*

22. *Atanassova V, Meindl A, Ring C. Prevalence of Staphylococcus aureus and staphylococcal entrotoxins in yaw pork and uncooked smoked ham: a comparison of classical culturing detection and RFLP-PCR. International journal of food microbiology 2001; 68: 105-113.*

23. *Sina H, Baba-Mousa F, Kayode AP, Kotchoni SO. Characterization of Staphylococcus aureus isolated from street foods: toxic profile and prevalence of antibiotic resistance. Journal of Applied Biosciense 2011; 46: 3133-3143.*

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Clinical analysis of diffuse interstitial lung disease patients with positive anti-neutrophil cytoplasmic antibody

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Abstract

Objective: To explore the features of pulmonary interstitial pathological changes in diffuse interstitial lung disease (DILD) patients with positive anti-neutrophil cytoplasmic antibody (ANCA), and the similarities as well as differences between ANCA-positive patients with non-primary vasculitis and primary systematic vasculitis.

Methods: 151 DILD patients with complete clinical data of ANCA admitted in our hospital from March 2004 to July 2012 were retrospectively analyzed. The results of syndromes, signs, radiological manifestations, pulmonary function tests, fiberoptic bronchoscope examinations, cytologic analysis of bronchoalveolar lavage fluid (BALF) and other laboratory examinations on ANCA-positive patients with non-primary vasculitis (Group A), those with primary systematic vasculitis (Group B) and ANCA-negative patients(Group C) were compared.

Results: In the 151 DILD patients with ANCA results, 45 patients' ANCA (29.80%) were positive. The numbers of patients in Groups A, B and C were 9, 36, and 106. Total lung capacity (TLC) decreased less and pleural pathological changes were more common in Groups A and B than Group C. Oliguria, haematuria, proteinuria, anaemia and renal inadequacy in Group A were similar to those in Group C, appeared less than in Group B. The results of fiberoptic bronchoscope examination, BALF cytology and anti-nuclear antibody (ANA) were not significantly different among the three groups.

Conclusion: In DILD patients, pulmonary interstitial pathological changes of those with positive ANCA were accompanied with more pleural pathological changes and TLC decreased less than those with negative ANCA. In patients with positive ANCA, non-primary vasculitis had some similar clinical manifestations to primary systematic vasculitis, however, anaemia and renal damages were relatively uncommon in the non-primary vasculitis patients.

Key words: Anti-neutrophil cytoplasmic antibody; interstitial lung disease; respiratory function; fiberoptic bronchoscope.

Introduction

Diffuse interstitial lung disease (DILD) [1] is a generic term of a large class of heterogeneous diseases mainly involving interstitial lung, including systemic diseases-related DILD (such as connective tissue disease, vasculitis, etc.), environment or drug-induced DILD, idiopathic interstitial pneumonia, granulomatous diseases (such as sarcoidosis, Wegener's granulomatosis, etc.), and other rare diffuse lung diseases. Although the etiology, pathogenesis, treatment and prognosis of DILD are different, the symptoms, signs and radiographic changes, pulmonary function impairment are similar. Currently, it is considered that anti-neutrophil cytoplasmic antibody (ANCA) participates in the formation of part of pulmonary interstitial diseases in DILD diseases [2]. ANCA, as a class of autoantibodies, is mainly relevant with primary systematic vasculitis (Wegener's granulomatosis, microscopic polyangiitis and allergic granulomatosis, a few of which can be found in other diseases, such as systemic lupus erythematosus, rheumatoid arthritis, etc. [3,4]. In the above-mentioned diseases, there is rare report on the clinical significance of ANCA in pulmonary interstitial diseases and the differences between the manifestations of non-primary vasculitis and primary systematic vasculitis in ANCA-positive patients. To this end, retrospective analysis was conducted on 151 DILD patients with unconfirmed diagnosis

who had the data of ANCA examinations admitted in our hospital from March 2004 to July 2012 so as to explore the above problems.

Materials and Methods

Objects

There were a total of 436 DILD patients treated in our hospital from March 2004 to July 2012, in which 151 patients had complete ANCA examination data, including 45 ANCA-positive cases and 106 ANCA-negative cases. The 6 patients with positive cytoplasmic ANCA (C-ANCA) were anti-proteinase 3 (PR3) antibodies positive, who were diagnosed as Wegener's granulomatosis; there were 33 patients with positive perinuclear ANCA (P-ANCA), including 30 cases of positive anti-myeloperoxidase (MPO) antibody (28 cases of microscopic polyangiitis and 2 cases of allergic granulomatosis respectively) and 3 cases of negative anti-MPO antibody (1 case of dermatomyositis and 2 cases of mixed connective tissue disease respectively); there were 6 patients with positive indirect immunofluorescence and negative anti-MPO and PR3 antibody, including 3 cases of dermatomyositis and 1 case of rheumatoid arthritis, systemic lupus erythematosus and acute interstitial pneumonia respectively. In the 106 ANCA-negative patients, 53 were diagnosed as idiopathic interstitial pneumonia (including 16 cases of idiopathic pulmonary interstitial fibrosis, 9 cases of nonspecific interstitial pneumonia, 4 cases of acute interstitial pneumonia, 2 cases of cryptogenic organizing pneumonia and 22 undifferentiated-typed cases), 15 rheumatoid arthritis, 10 dermatomyositis, 7 Sjogren's syndrome, 3 scleroderma, 2 systemic lupus erythematosus, 4 mixed connective tissue disease and 12 unclassified connective tissue disease.

The diagnosis of primary systemic vasculitis met the vasculitis classification criteria Chapel Hill developed in 1999 [5]. Both Wegener's granulomatosis and allergic granulomatosis were confirmed by pathological diagnosis; for microscopic polyangiitis, 21 cases were pathologically confirmed, and 7 cases were the clinical diagnosis excluding other diseases according to the lung and kidney damage associated with autoantibodies examination results. The diagnosis of idiopathic interstitial pneumonia conformed to the interstitial lung disease classification criteria jointly formulated by the American Thoracic Society and European Respiratory Society in 2002 [6], of which there were 27 cases of pathological confirmation (including 17 cases of open lung biopsy and 10 cases of bronchoscopic lung biopsy) and 27 cases of clinical diagnosis excluding secondary interstitial lung diseases caused by other etiologies according to the clinical manifestations and radiological abnormalities of patients, combining with the results of autoantibodies and BALF examinations. The diagnosis of a variety of connective tissue diseases accorded with the standards for various diseases developed by the Chinese Rheumatology Association (CRA) [7], in which all cases of scleroderma, 12 cases of dermatomyositis, 4 cases of Sjogren's syndrome and 1 case of systemic lupus erythematosus were pathologically confirmed, and 3 case of dermatomyositis, 4 cases of Sjogren's syndrome and 1 case of systemic lupus erythematosus were clinical diagnosis excluding other diseases according to the multi-system damage, blood serum muscle enzymes and autoantibodies examination; rheumatoid arthritis, mixed connective tissue disease and unclassified connective tissue disease were the clinical diagnosis excluding other diseases based on the articular and skin manifestations, combined with autoantibodies examination. All these patients were divided into three groups: non-primary vasculitis ANCA-positive group (A), primary systematic vasculitis ANCA-positive group (B) and ANCA-negative group (C).

Methods

The differences in clinical symptoms, signs, lung function indicators, radiological manifestations (chest CT), blood gas analysis, fiberoptic bronchoscope examinations, BALF total cellular score and differential counting as well as other laboratory examinations were compared among Groups A, B and C.

Statistical analysis

The statistical analysis was made by the statistical software of SPSS 17. 0. The one-way ANOVA was adopted for the comparison of mean among the three groups of samples, and the Chi-square test for the rate comparison, $P<0.05$ for statistically significant difference.

Results

General information

There were 9 patients in Group A, including 5 males and 4 females, aged from 49 to 82 years old, with the average age of (62.19±12.89) years old; 36 patients of Group B included 18 males and 18 females, of whom the oldest was 77 years old, the youngest 32 years old, and the average age (62.01 ± 13.31) years old; 106 patients of Group C included 54 males and 52 females, of whom ages were from 27 to 87 years old, (61.77±12.59) years old on average; the age differences among the three groups were not statistically significant (P>0.05). The courses of disease of the three groups were 0.1 to 15.25 years, 0.1 to 27.34 years and 0.25 to 20.1 years, with the median of 4 months, 8 months and 9 months respectively; the time from onset of symptoms to diagnosis was 1 month to 14 years, 1 month to 28 years and 3 months to 18 years respectively, with the median of 5 months, 9 months and 9 months; the ages of onset were respectively (62.55 ± 12.48) years old, (60.83 ± 15.54) years old and (60.17 ± 14.13) years old; in the three groups, there were 3 patients, 11 patients and 41 patients smoking respectively, with the smoking index of (10.03 ± 10.89) year/package, (17.23 ±15.14) year/package and (18.87 ± 14.55) year/package, among which the differences were not statistically significant (P> 0.05). 6 patients in Group C had a history of dust exposure, with the time from 9 to 34 years, while there was no clear history of occupational exposure in Groups A and B (Table 1).

Symptoms and signs

1 patient in Group A was diagnosed as bilateral pleural effusion, and there were 4 cases of pleural effusion in Group B, in which 2 cases were bilateral and 2 cases right-sided; eye damages mainly included blurred vision, uveitis, ocular pain and swelling; skin lesions were manifested as maculopapular rash, redness and pigmentation in limbs, trunk or face, hard and swollen acral skin, scaling and Raynaud's phenomenon in both hands; muscle damages were mainly shown in sore and

Table 1. General information ($\bar{x} \pm s$)

Item		Group A (n=9)	Group B (n=36)	Group C (n=106)
Gender	Male	5	18	54
	Female	4	18	52
Age (years old)		62. 19 ±12. 89	62. 01 ±13. 31	61. 77±12. 59
Onset age (years old)		62. 55±12. 48	60. 83±15. 54	60. 17±14. 13
Disease course (year)		0.1-15.25	0.1-27.34	0.25-20.1
Smoking	Smoking case No.	3	11	41
	Smoking index (bag/year)	10. 03±10. 89	17. 23±15. 14	18. 87±14. 55
Dust exposure case No.		-	-	6

Table 2. Main symptoms and signs

Item	Group A (n=9)	Group B (n=36)	Group C (n=106)	P value
Fever	6 (66.67%)	22 (61.11%)	54 (50.94%)	0.376
Cough	9 (100.00%)	29 (80.56%)	90 (84.90%)	0.389
Breathe hard	8 (88.89%)	19 (52.78%)	86 (81.13%)*	0.019
Rhonchi Lung Sound	8 (88. 89%)	35 (97. 22%)	95 (89. 62%)	0. 461
Pleural effusion	1 (11.11%)	4 (11. 11%)	0 (0. 00%)	-
Na Cha	4 (44. 44%)	21 (58. 33%)	38 (35. 84%)	0. 102
Oliguria	0 (0. 00%)	5(13. 89%)	2 (1. 89%)	-
Edema	3 (33.33%)	12 (33.33%)	15 (14.15%)*	0.045
Skin injury	3 (33. 33%)	7(19. 44%)	18 (16. 98%)	0. 713
Joint injury	1 (11. 11%)	4 (11. 11%)	28 (26. 42%)	0. 159
Eye lesion	0 (0. 00%)	5 (13. 89%)	4 (3. 77%)	-
Muscle lesion	2 (22. 22%)	3 (8. 33%)	11 (10.37%)	0. 785

** P < 0. 05, compared to Group B*

fatigue limbs or muscles; joint damages mainly included multi-joint swelling and pain, deformity, morning stiffness (Table 2).

Laboratory examination

All the patients in this study underwent routine examinations on peripheral blood hemoglobin, urine, erythrocyte sedimentation rate (ESR), serum creatinine (Cr), serum urea nitrogen (BUN), C-reactive protein (CRP), rheumatoid factor (RF), antinuclear antibodies (ANA) and anti-extractable nuclear antigen antibodies (ENA), the results of which are shown in Table 3.

Imaging results

136 patients received pulmonary CT examination, including 9 cases in Group A, 30 cases in Group B and 97 cases in Group C (Table 4). In the three groups, patchy shadow and reticular shadow were mostly bilateral pulmonary multiple changes, and their lesion distributions were similar, manifested with diffused distribution in bilateral lung or mainly in inferior peripheral pleura or bilateral lower lung, and in addition, a few were limited distribution. 6 patients in Group B showed honeycomb changes, similar to the manifestations of idiopathic pulmonary fibrosis (i.e. distributed near to dual lower lung near pleura), and 1 patient was manifested with irregular distribution in bilateral lung; there were 18 patients in Group C manifested with idiopathic interstitial pulmonary fibrosis, 6 cases diffused distribution in bilateral lung and 1 case irregular distribution. For patients with ground-glass opacity, 1 case in Group A showed bilateral pulmonary multiple change; while there were 3 and 16 cases having this change in Groups B and C respectively. In these two groups, there were also bilateral lower lung distribution in 10 cases and peripheral subpleural distribution in 15 cases respectively. Conglomerate shadow was only seen in a patient with Wegener's granulomatosis in Group B.

Fiberoptic bronchoscopy and lavage fluid analysis

69 patients were conducted with fiberoptic bronchoscopy, including 3 in Group A, 15 in Group B and 51 in Group C. 1 case, 5 and 25 cases in

Table 3. Laboratory examination results

Item	Group A (n=9)	Group B (n=36)	Group C (n=106)	P value
Anemia	1 (11. 11%)*	22 (61. 11%)	4 (3. 77%)*	< 0. 01
Hematuria	0 (0. 00%)	19 (52. 78%)	10 (9. 43%)	-
Albuminuria	1 (11. 11%)*	18 (50. 00%)	12 (11. 32%)*	< 0. 01
BUN increase	1 (11. 11%)*	15 (41. 67%)	7 (6. 60%)*	< 0. 01
Cr increase	0 (0. 00%)	17 (47. 22%)	9 (8. 49%)	-
ESR increase	7 (88. 89%)	29 (80.56%)	78 (73. 58%)	0. 705
CRP increase	5 (55. 56%)	20 (55. 56%)	65 (61. 32%)	0. 816
RF positive	2 (22. 22%)	18 (50. 00%)	44 (41. 51%)	0. 467
ANA positive	5 (55. 56%)	15 (41. 67%)	57 (53. 77%)	0. 489
ENA positive	1 (11.11%)	2 (5. 56%)	22 (20. 75%)	0. 221

** P < 0. 01, compared to Group B*

Table 4. Imaging results

Imaging change	Group A (n=9)	Group B (n=30)	Group C (n=97)	P value
Patchy shadow	8(88. 89%)	14 (58. 33%)	49(50. 51%)	0. 181
Reticular change	5(55. 56%)	11 (45. 83%)	67 (69. 07%)	0. 096
Honeycomb	0 (0. 00%)	7 (23. 33%)	25 (25. 77%)	-
ground-glass opacity	1 (11. 11%)	13 (43. 33%)	31 (31. 96%)	0. 112
Conglomerate shadow	0 (0. 00%)	1 (3. 33%)	0 (0. 00%)	-
Hilus pulmonis or mediastinal lymphadenectasis	4 (44. 44%)	11(36. 67%)	47 (48. 45%)	0. 617
Pleural change	5(55. 56%)*	5(16. 67%)*	3 (3. 09%)	< 0. 01

** P < 0. 01, compared to Group C*

the three groups respectively were bronchoscopic normal, and 2, 10 and 26 cases showed inflammation respectively (mucosal congestion, edema and serous secretions). 1 patient in Group B was manifested with granulomatous lesions scattered in main bronchus and bilateral bronchial wall at all levels. There were 3, 7 and 42 patients undergoing routine BALF analysis respectively in the three groups, in which 2, 3 and 37 were observed increased percentage of neutrophile granulocytes, and 1, 3 and 28 elevated lymphocyte percentage in the lavage fluid respectively.

Pulmonary function and blood gas analysis

Pulmonary function

In Groups A, B and C, 6, 17 and 78 patients received pulmonary function examination respectively, in which there were 4, 6 and 44 cases of restrictive ventilatory dysfunction; 0 case, 3 cases and 1 case of obstructive ventilatory dysfunction; 4, 12 and 56 cases of diffuse dysfunction; 1 case, 0 case and 3 cases of small airway dysfunction; 0 case, 1 case and 4 cases of mixed ventilatory dysfunction, as well as 0, 1 and 6 normal cases respectively. The differences in the proportion of abnormal lung function types were not statistically significant among the three groups (P> 0 05). The percentages of total lung capacity (TLC) were (83.257 ± 11.532)% and (86.734 ± 13.597)% respectively in Groups A and B, significantly higher than that of Group C [(72.931 ±18.561)%, P = 0.048]; there were no statistically significant differences in the other indicators (FEV 1%, FEV1 / FVC, VC%, RV%, DLCO%, FEF 25-75%, Vmax75%, Vmax50%, Vmax25%) among the three groups.

Blood gas analysis

9, 30 and 98 patients were made blood gas analysis respectively in Groups A, B and C. The results showed that there were 6, 18 and 57 cases of hypoxemia (PO_2 <80 mmHg, 1 mmHg = 0.133 kPa) and 4, 15 and 40 cases of type I respiratory failure (PO_2 <60 mmHg) respectively; the PO_2 values of the three groups were (71.546 ± 19.132) mmHg, (73.836 ± 23.195) mmHg and (74.019 ± 18.716) mmHg, and PCO_2 values (36.126 ± 4.068) mm Hg, (36.258±7.682) mm Hg and (37.169 ± 5.173) mmHg respectively, showing no statistically significant differences (P> 0.05).

Discussion

In diffuse interstitial lung disease (DILD), although the etiology, pathogenesis, treatment and prognosis of connective tissue disease correlative DILD, primary vasculitis and idiopathic interstitial pneumonia are different, radiological changes and lung damage of the diseases were similar. It has been reported that in the above-mentioned diseases, ANCA may be involved in the formation of part of pulmonary interstitial inflammation and fibrosis [8], which as a class of autoantibodies with neutrophil cytoplasmic and monocyte cytoplasmic constituents as target antigen, promotes the activation and chemotaxis of inflammatory cells by binding with the target antigens (such as MPO, PR3, etc.) to produce reactive oxygen species so as to result in target cell injury, damage medium and small blood vessels and capillary wall to form fibrinoid lesions, accompanied or not accompanied by the occurrence of necrotizing granuloma, luminal stenosis or occlusion, involving the lung, kidney and other organ systems [9]. ANCA is mainly associated with primary systematic vasculitis, a few of which can also be found in other diseases. In this study, ANCA-positive cases of non-primary vasculitis are mostly indirect immunofluorescence antibody positive, ELISA antibody (anti-PRO3 and anti-MPO antibodies) negative. The most common pulmonary symptoms and signs of interstitial lung disease patients with positive or negative ANCA are cough, expectoration, shortness of breath and pulmonary rales (fine rales and crackles); nonspecific systemic symptoms (fever, anorexia, weight loss, etc.), as well as damages in kidney, skin, muscles, joints and other extrapulmonary systems, consistent with literature reports [10]. In the 45 ANCA-positive patients, there were 5 cases of pleural effusion, while this symptom was not found in 106 patients with negative ANCA, which suggests that thoracic pathological changes participated by ANCA may be more often involved the pleura, which is in line with relatively common pleural lesions in imaging in such patients. In ANCA-positive patients, the proportion of the occurrence of oliguria in non-primary vasculitis was similar to that of ANCA-negative patients, both of which were significantly less than that of primary systemic vasculitis, indi-

cating whether the patients were ANCA positive or not, the renal involvement of non-primary vasculitis is less than that of primary vasculitis. In the three groups of patients, ANCA-positive patients with primary vasculitis who suffered from edema were significantly more than those with negative ANCA, showing no significant differences among the groups. The causes for the formation of edema are relatively complicated, which may be relevant with vascular inflammation, retal damage and many other factors, therefore, further studies with large samples are needed on whether ANCA is associated with edema or not.

In this study, anemia, hematuria, proteinuria and renal insufficiency in ANCA-positive patients with non-primary vasculitis were similar to those in patients with negative ANCA, both of which were significantly rare compared with primary vasculitis patients, suggesting that regardless of positive or negative ANCA, renal involvement in non-primary vasculitis is less common than that of primary systemic vasculitis. There were no significant differences in ESR, CRP, RF and ANA abnormalities among the three groups, so it was non-specific for the diagnosis of etiologies, which should be combined with other examinations for comprehensive analysis.

Currently, it is believed that a variety of imaging abnormalities (such as patchy shadow, reticular shadow, and ground-glass opacity, etc.) can be seen in DILD with different etiologies [11]. In this study, the most common pulmonary pathological changes of ANCA-positive and -negative patients were patchy shadow or reticular shadow, mainly bilateral pulmonary multiple changes. For patients with positive ANCA, pulmonary interstitial change, alveolar inflammation, pleural disease and many other abnormalities often occurred simultaneously, in which pleural lesion was significantly more than that of ANCA-negative patients. It has been reported that patients with ANCA-positive systemic lupus erythematosus [12] are more likely to have pleural effusion and other pleural involvement manifestations compared with those with negative ANCA. However, in primary vasculitis, rheumatoid arthritis, dermatomyositis and other diseases, the pleural disease of ANCA-positive patients is more significant than that of those with negative ANCA, which has not yet reported in literatures. Therefore, whether ANCA is relevant with the occurrence of pleural diseases still needs to be confirmed by further studies. ANCA-positive patients whose pulmonary interstitial lesions showed patchy shadow (22/45) seemed to be more than ANCA-negative patients (49/106), with the distribution of lesions similar to the latter; the proportion of the emergence of ground-glass opacity and reticular shadow in ANCA-positive patients was similar to that in ANCA-negative patients, but in the distribution of lesions, ground-glass opacity of ANCA-positive patients were mostly located in bilateral lower lung or inferior peripheral pleura (9/14), which seemed to be different from that in ANCA-negative patients (14/31), but their distributions of reticular shadow were similar. It is generally believed that honeycomb change is rare in primary vasculitis [13], in which lesions form into ANCA to prompt the production of macrophages and the increased release of basic fibroblast growth factor (e.g. fibronectin), resulting in the abnormal proliferation of fibroblasts to produce collagen, which causes the occurrence of irreversible fibrosis or recurrent subclinical alveolar hemorrhage, so as to eventually lead to interstitial fibrosis [14,15]. In this study, the ANCA-positive patients had fewer honeycomb lungs (7/39) than those with negative ANCA (25/97), but the proportion of honeycomb lung in primary vasculitis patients was similar to those with negative ANCA, different from the literature reports, which may be related to the relatively few number of cases and high proportion of collagen vascular disease and usual interstitial pneumonia (UIP) in ANCA-negative cases. The image distribution of honeycomb changes in ANCA-positive patients with primary vasculitis was similar to that of ANCA-negative patients, which was mostly UIP manifestation, and was also irregularly distributed, accompanied by patchy shadow, reticular shadow and other changes, but the former is often combined with renal insufficiency and anemia clinically, which was significantly different from ANCA-negative patients, so routine ANCA examinations should be performed on pulmonary fibrosis patients with unconfirmed diagnosis. If patients suffer from positive ANCA combined with renal damage, primary vasculitis should be considered first. Conglomerate shadow is considered to be the most common image ma-

nifestation of Wegener's granulomatosis [16]. In this study, regardless of ANCA-positive or-negative patients, no similar change was found in other diseases causing interstitial lung diseases, therefore, if pulmonary conglomerate shadow occurs in DILD patients with positive ANCA , Wegener's granulomatosis [17] should be considered first.

In this study, the lung function abnormalities were mainly manifested in that both ANCA-positive and-negative patients mainly showed diffusion impairment and restrictive ventilatory dysfunction, which suggested that similar pathophysiological changes existed in both, that is, alveolar inflammation causes the integrity failure of alveolar wall, so that decreased gas exchange area and pulmonary interstitial fibrosis lead to the degeneration of lung compliance. As for whether the lung function indicators of ANCA-positive and-negative patients differs [18], previous studies were mainly limited to patients with pulmonary interstitial fibrosis changes [19], which found that FEV1%, TLC%, DLCO% and other indicators showed no significant differences between the two groups; while for patients with other types of lung abnormalities, whether there is difference between the two remains uncertain. In this study, no significant differences were found in the airflow indicators (FEV1%, FVC%, etc.) between ANCA-positive and -negative patients, and TLC of the former declined significantly less than that of the latter. This may be because the inflammatory process ANCA participates in is mainly small vessel involvement, which can directly result in relatively light alveolar wall damage and formation of interstitial fibrosis pathological changes, and thus it has a relatively small impact on lung tissue compliance.

However, the lung function indicators may be affected by a wide range of complicated factors, such as varieties of primary diseases and interstitial pathological changes, disease severity and duration, etc. [20]. Therefore, further more detailed explorations are needed for how ANCA influences the abnormal change of lung function and whether the differences in lung function indicators of ANCA-positive and-negative patients are relevant with different types of radiological abnormalities. In addition, a patient's age, occupational factors and smoking can also affect his/her lung function levels, but in the three groups of patients in this study, there were a very small number of cases with a history of occupational exposure, and the age, the proportion of number of smokers as well as smoking index were similar, so these were not the main reasons for the differences in lung function among the groups [21]. Currently, there are few studies on the manifestations of DILD patients with positive ANCA via fiberoptic bronchoscope. This study showed that airway mucosal congestion and edema, serous or white viscous secretion and other changes in the lumen of ANCA-positive and-negative patients could be observed via fiberoptic bronchoscope; in the primary vasculitis group, a case diagnosed as Wegener's granulomatosis had scattered mucosal granulomatous lesion on the airway, which was a characteristic manifestation of this disease, consistent with the literature [22]. The above findings suggest that, except relatively obvious characterized manifestations of some cases, the airway of DILD patients has no characteristic presentations, and is not entirely consistent with the severity of lung diseases, which is of no great significance to the implications for diagnosis [23]. Generally, BALF has important significance for the finding of concealed alveolar hemorrhage in ANCA-associated diseases [24], while for patients without alveolar hemorrhage, BALF cytological classification is not characteristic [25]. In this study, whether the patients were ANCA positive or not, the proportion of lymphocytes or neutrophils in BALF cell classification were increased, consistent with previous reports.

In summary, in DILD, patients with ANCA-positive interstitial lung disease combined pleural involvement are relatively common, and TLC reduces less than those with negative ANCA. For patients with positive ANCA, non-primary vasculitis and primary systematic vasculitis have similar clinical manifestations, but combined renal damage and anemia are relatively rare. Therefore, patients with interstitial lung disease should be paid close attention to track and detect the ANCA and extrapulmonary clinical manifestations in the course of the disease.

References

1. *Patiwetwitoon S, Wangkaew S, Euathrongchit J, Kasitanon N, Louthrenoo W. High-resolution computed tomographic findings in systemic sclerosis-associated interstitial lung disease: comparison between diffuse and limited systemic sclerosis. J Clin Rheumatol 2012; 18: 229-233.*

2. *Tanaka T, Otani K, Egashira R, Kashima Y, Taniguchi H, Kondoh Y, et al. Interstitial pneumonia associated with MPO-ANCA: Clinicopathological features of nine patients. Respir Med 2012; 106: 1765-1770.*

3. *Reinhold-Keller E. Diagnostics and Therapy of Antineutrophil Cytoplasmic Antibody (ANCA) Associated Vasculitides. Curr Pharm Des 2012; 18: 4537-4541.*

4. *Bhamra K, Luqmani R. Damage Assessment in ANCA-Associated Vasculitis. Curr Rheumatol Rep 2012; 14: 494-500.*

5. *Jennette JC, Falk R J, Andrassy K, et al. Nomenclature of systemic vasculitis: The proposal of an international consensus conference. Arth Rheum1994; 37(2) : 187.*

6. *Katzenstein AL, Myers JL. Nonspecific interstitial pneumonia and the other idiopathic interstitial pneumonias: classification and diagnostic criteria. Am J Surg Pathol 2000; 24: 1-3.*

7. *Dong Y, Zhao Y, Guo X. [Preliminary diagnostic criteria for primary Sjogren's syndrome in China]. Zhonghua Nei Ke Za Zhi 1996; 35: 114-117.*

8. *Yamazaki C, Arai S, Tamura Y, Suzuki Y, Nakajima H, Kojima K, et al. [Case of rapidly progressive glomerulonephritis with anti-glomerular basement membrane antibody in the course of MPO-ANCA-associated pachymeningitis]. Nihon Jinzo Gakkai Shi 2009; 51: 490-495.*

9. *Chhabra S, Minz RW, Goyal L, Singh S. Atypical ANCA pattern with simultaneous presence of MPO and PR3-ANCA in systemic lupus erythematosus. J Postgrad Med 2010; 56: 217-218.*

10. *Chen DH, Lin YN, Lan SL, Pan XA, Zeng QS, He ZT, et al. [Clinical characteristics of bronchiolitis obliterans in pediatric patients]. Zhonghua Er Ke Za Zhi 2012; 50: 98-102.*

11. *Boland JM, Tazelaar HD, Colby TV, Leslie KO, Hartman TE, Yi ES. Diffuse pulmonary lymphatic disease presenting as interstitial lung disease in adulthood: report of 3 cases. Am J Surg Pathol 2012; 36: 1548-1554.*

12. *Caltik A, Demircin G, Bulbul M, Erdogan O, Akyuz SG, Arda N. An unusual case of ANA negative systemic lupus erythematosus presented with vasculitis, long-standing serositis and full-house nephropathy. Rheumatol Int 2010.*

13. *Wiszniewska M, Szylberg T, Harat M. [Primary central nervous system vasculitis imitating a brain tumour--a case report]. Neurol Neurochir Pol 2008; 42: 358-361.*

14. *Bob FR, Gluhovschi G, Herman D, Potencz E, Gluhovschi C, Trandafirescu V, et al. Histological, immunohistochemical and biological data in assessing interstitial fibrosis in patients with chronic glomerulonephritis. Acta Histochem 2008; 110: 196-203.*

15. *Siomou E, Tramma D, Bowen C, Milford DV. ANCA-associated glomerulonephritis/systemic vasculitis in childhood: clinical features-outcome. Pediatr Nephrol 2012; 27: 1911-1920.*

16. *Horger M, Goeppert B, Hartmann J, Vogel M. [Imaging findings in Wegener's granulomatosis]. Rofo 2007; 179: 445-449.*

17. *Stahelin L, Fialho SC, Neves FS, Junckes L, Werner de Castro GR, Pereira IA. Cocaine-induced midline destruction lesions with positive ANCA test mimicking Wegener's granulomatosis. Rev Bras Reumatol 2012; 52: 431-437.*

18. *Nozu T, Kondo M, Suzuki K, Tamaoki J, Nagai A. A comparison of the clinical features of ANCA-positive and ANCA-negative idiopathic pulmonary fibrosis patients. Respiration 2009; 77: 407-415.*

19. *Homma S, Matsushita H, Nakata K. Pulmonary fibrosis in myeloperoxidase antineutrophil cytoplasmic antibody-associated vasculitides. Respirology 2004; 9: 190-196.*

20. *Ostrowski S, Barud W. Factors influencing lung function: are the predicted values for spirometry reliable enough? J Physiol Pharmacol 2006; 57 Suppl 4: 263-271.*

21. *Seo T, Pahwa P, McDuffie HH, Yurube K, Egoshi M, Umemoto Y, et al. Association between cytochrome P450 3A5 polymorphism and the lung function in Saskatchewan grain workers. Pharmacogenet Genomics 2008; 18: 487-493.*

22. *Mohammad AJ, Segelmark M. Association of cigarette smoking with organ damage in primary systemic vasculitis. Scand J Rheumatol 2011; 40: 51-56.*

23. *Cano-Jimenez E, Molina-Molina M, Ramirez J, Sanchez M, Aliaga JL, Xaubet A. [Diffuse interstitial*

lung disease related to peribronchiolar metaplasia]. Arch Bronconeumol 2009; 45: 57-59.

24. *Cobanoglu N, Ozcelik U, Cetin I, Yalcin E, Dogru D, Kiper N, et al. Anti-neutrophil cytoplasmic antibodies (ANCA) in serum and bronchoalveolar lavage fluids of cystic fibrosis patients and patients with idiopathic bronchiectasis. Turk J Pediatr 2010; 52: 343-347.*

25. *Itoh M, Oh-Ishi S, Nemoto K, Senba S, Adachi H, Kishi K, et al. A case of diffuse alveolar hemorrhage associated with tegafur plus uracil and warfarin therapy. Clin Med Insights Case Rep 2011; 4: 73-77.*

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Gemcitabine cisplatin combination in locally advanced and metastatic non small cell lung cancer: Single center experience

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Abstract

Objective: To determine responses to Gemcitabine-Cisplatin combination and toxicities of the locally advanced and metastatic Non Small Cell Lung Cancer cases monitored at our clinic.

Design: Retrospective study

Setting: Antalya Education and Research Hospital, Department of Medical Oncology, Antalya, Turkey

Subjects: The patients with locally advanced and metastatic Non Small Cell Lung Cancer

Interventions: The chemotherapy combination consisting of Gemcitabine 1250 mg/m2 for the days 1+8 and Cisplatin 75 mg/m2 for the day 1 has been applied every 21 days.

Main Outcome Measure: Treatmant response and survival

Results: Total 51 patients, of which 10 (19.6 %) were female and 41 (80.4%) have been involved in the study. Response to Gemcitabine-Cisplatin combination was obtained in total 29 (56.8%) patients, of which 24 (47%) were partial response and 5 (9.8%) were stable disease. Average survival of the patients was determined as 14,4 months and median survival as 10,7 months (95% confidence interval 6,4-14,9)

Conclusion: Consequently, Gemcitabine-Cisplatin combination is an efficient treatment alternative with tolerable toxicity in advanced and metastatic phase NSCLC. Determination of the factors which may impact this response will protect some patients from the complications of treatment and will prevent losing some patients due to treatment-related reasons.

Key words: Lung Cancer, Gemcitabine, Cisplatin, Cheomothreapy.

Introduction

Lung cancer is the most common cause of mortalities from cancer. Lung cancer has two basic histological types being small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC) and NSCLC consists 80-85% of all cases (1). Half of all NSCLC cases are in advanced or metastatic phases during diagnosis. In these patients, cure cannot be obtained and standart treatment is chemotherapy for the patients with good performance status (2). The treatment of lung cancer can vary with respect to the cell type, tumor phase, biological characteristics of the tumor (such as Epidermal Growth Factor Receptor (EGFR), Anaplastic Lymphoma Kinase (ALK) expression) and overall performance of the patient (3).

For NSCLC, Gemcitabine, Paclitaxel, Docetaxel, Vinorelbine are the effective drugs (4-6). Combination with Cisplatin of these agents is more successful and they are commonly used because of their impacts on survival, although moderate (2). In the first stage treatment of NSCLC, it is controversial which treatment combination should be preferred and advantage of one combination over the other could not be shown (7).

The objective of this study is to determine responses to Gemcitabine-Cisplatin combination and toxicities of the locally advanced and metastatic NSCLC cases monitored at our clinic.

Material and method

The Patients

The patients with locally advanced and metastatic NSCLC who has got the diagnosis of NSCLC between 2008-2010 at Antalya Education and Research Hospital, Department of Medical Oncology

and whose staging studies have been conducted by screening methods, have been evaluated. The patients at phase IIIB and IV according to the 7th staging system of The American Joint Committee on Cancer (AJCC) have been evaluated. The performance scores of the cases have been evaluated according to the scoring system of Eastern Cooperative Oncology Group (ECOG). Those ECOG 0-2 patients with objectively measurable disease, having sufficient bone marrow reserve, having normal hepatic and renal function have been involved in the study. The patient files have been searched and the information about the age, gender, stage of the disease and the treatments received have been gathered. For response evaluation, RECIST response criteria have been used (8).

Treatment

The chemotherapy combination consisting of Gemcitabine 1250 mg/m2 for the days 1+8 and Cisplatin 75 mg/m2 for the day 1 has been applied every 21 days. The body surface was calculated with DuBois formula (BSA: (Weight ^{0,425} x Height ^{0,725})x 0,007184). Gemcitabine-Cisplatin combination was applied as the first stage treatment in all patients. Following the second cycle, the response of the patients was evaluated. For the patients with response, the treatment was completed to 6 cycles. Prior to each cycle, complete blood count, liver and kidney function tests and electrolytes have been evaluated. Chemotherapy dependent toxicities were evaluated with respect to the toxicity criteria of World Health Organization.

Statistical Analysis

SPSS 15.0 software was utilized in statistical analyses. Examination of survival with univariate analyses has been performed by log rank test. The independent factors for estimating survival were examined utilizing Cox regression analysis with backward selection method using the multivariate analyses and the possible factors determined in the previous analyses. Survival rates were examined with Kaplan Meier survival analysis. Those cases having type 1 failure level of less than 5% were interpreted as statistically significant.

Results

Total 51 patients, of which 10 (19.6%) were female and 41 (80.4%) have been involved in the study. Patient age was determined as 60,6±11,7 (Table 1). About 80% of the patients had the history of smoking for more than 10 years. While smoking proportion was 30% for females, it was determined as 90% for males.

Most frequent application symptoms of the patients were cough (88.2%) and dyspnea (54.9%). While wedge resection has been used in 5 patients and lobectomy has been used in 2 patients, the other patients have been diagnosed by bronchoscopic biopsy. Sixteen patients (31.4%) were Stage IIIB, 35 patients (68.6%) were Stage IV. Performance status of the patients were between ECOG 0 and 2. Radiotherapy was used in 16 (31.4%) patients. Squamous cell carcinoma was determined in 27 patients (52.9%), adenocarcinoma in 20 (39.2%) patients, large cell carcinoma in 1 (2%) patient, and no histological subtype has been determined in 3 (5.9%) patients. While adenocarcinoma was deter-

Table 1. Patient Characteristics

	Mean, Standard Deviation	Median
AGE	60,6 ± 11,7	60
BOY (CM)	163,1 ± 8,6	164
BUN (MG/DL)	15,9 ± 5,1	16
CRE (MG/DL)	0,88±0,2	0,9
AST (U/L)	20,9 ± 10,6	19
ALT (U/L)	22,18± 18,2	18
LDH (U/L)	211,9±104,5	196
WBC (10^3/mm^3)	6,787±4,013	6,274
PLT (10^3/mm^3)	333,8±179,5	279
HB (G/DL)	12,7 ± 1,3	11,2

mined in 90% of the female patients, this proportion was 30% for male patients. Bones were the most common places of metastasis and determined in 13 (25.5%) patients. Other places of metastasis were the brain (7.8%), brain and bone (3.9%), liver (7.8%). Chemotherapy for total 206 cycles, median 4 cycles (between 2-6) was given to patients. Mostly encountered side effect was Grade 1-2 nausea and vomiting. As Grade 3-4 side effects, neutropenia in 6 (14.6%) patients, thrombocytopenia in 3 (7.3%) and anemia in 5 (12.1%) were determined. With one patient in whom renal toxicity was determined, the treatment was continued with Carboplatin.

Response to Gemcitabine-Cisplatin combination was obtained in total 29 (56.8%) patients, of which 24 (47%) were partial response and 5 (9.8%) were stable disease. While progression was determined in 13 (25.5%) of the patients, 7 patients were lost before evaluation of response and 2 patients went out of monitoring.

Mean survival of the patients was determined as 14,4 months and median survival as 10,7 months (95% confidence interval 6,4-14,9) (Figure 1). No significant relationship between the histological subtype and survival was determined (p:0.392). Significant relationship between gender and the survival was determined (p:0.006). While median survival was 25,7 months in females, it was 8,4 months in males (Figure 2). The relationship with survival and age, gender, cycles of chemotherapy, histological subtype, which are among the factors which may impact survival, was examined by Cox regression analysis. While statistically significant relationship was determined between the number of cycles of chemotherapy given to patients (p<0.001), gender (p:0.007) and survival, no statistically significant relationship was determined between patient's being older than 65, histological subtype, initial application symptoms and survival.

Table 2. Application symptoms of the patients

	Number of Patients	Proportion
Hoarseness	2	3.9%
Hemoptysis	9	17.6%
Dyspnea	28	54.9%
Cough	45	88.2%
Neurological Deficit	5	9.8%
Anorexia	14	27.5%
Weight Loss	27	54%

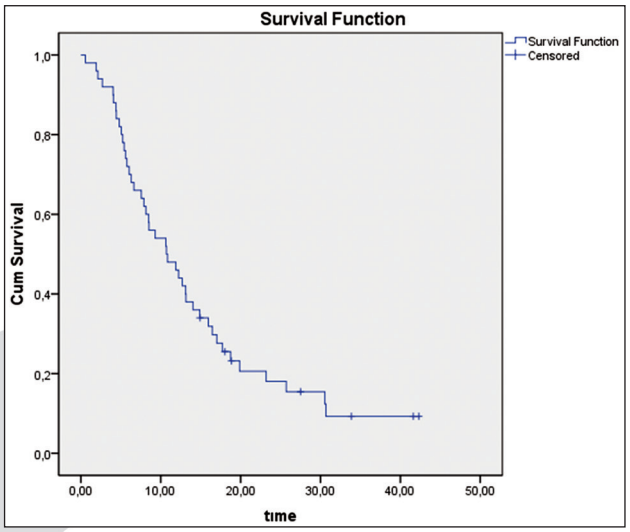

Figure 1. Survival curve of all patients.

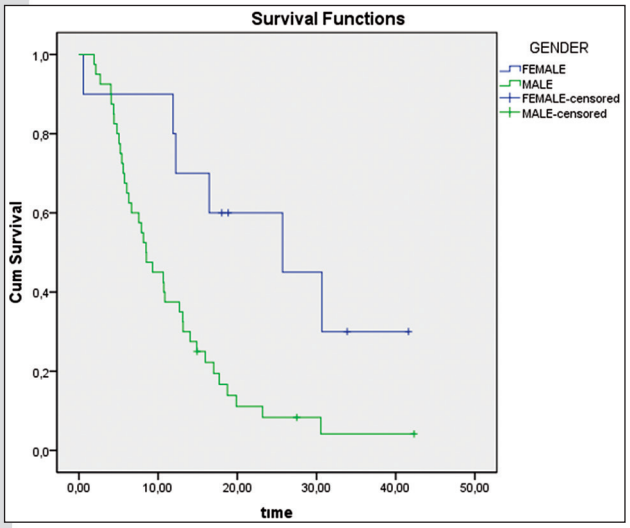

Figure 2. The graph of survival with respect to gender

Discussion

Gemcitabine is a deoxidine analog and although it structurally resembles Ara-C, its in cell pharmacology is different than Ara-C. It effects as a nucleotide analog by tranfsorming into active metabolyte inside the cell and is specific to S phase of the cell (9). In in-vitro studies, its efficacy on many tumours, NSCLC being in the first place, has been demonstrated (10, 11). With use of Gemcitabine as single agent, 20-25 % response percentage and 9 months of median survival have been obtained in NSCLC cases not given chemotherapy previously (11,13). In phase II studies where the combination of Gemcitabine with Cisplatin was evaluated, response percentages be-

tween 40 and 50% have been determined (14-16).

With use of Gemcitabine-Cisplatin combination at the first stage, average 14,1 months survival and 56.8% response were obtained. While the number of chemotherapy cycles was being impacted by the gender, no relationship between histological subtype, age's being older than 65, initial application symbols and survival was determined.

In sub-group analyses of ECOG 1594 study, it has been demonstrated that female gender has a survival advantage of 1.9 months over the male gender. In this study, while adenocarcinoma percentage was determined as 63% in female patients and 53% in male patients, smoking has not been evaluated (17). In our study, adenocarcinoma percentage has been determined as 90% in female patients and proportion of smoking was less than males. It is known that, in NSCLC, having or not having the history of smoking causes difference in survival (18). We think that the difference in survival of genders is associated with smoking status. We think that, histological subtype does not cause this difference. Because in NSCLC, histological subtype does not create survival difference in platin-based chemotherapies (19).

Schiller et al. have compared the efficacies of Cisplatin-Paclitaxel, Cisplatin-Gemcitabine, Cisplatin-Docetaxel and Paclitaxel-Carboplatin chemotherapy combinations. Superiority of any of these four combinations over the others could have not been shown. In this study, median survival in the Gemcitabine-Cisplatin group has been determined as 8.1 months. When the response is evaluated, partial response has been determined as 21%, stable disease as 18% and total response as 39% (7).

In a study where Vinorelbine Cisplatin combination was compared with Gemcitabine-Cisplatin, Paclitaxel-Cisplatin combinations, it has been shown that efficacies of the three regimens were similar. In Gemcitabine-Cisplatin branch of this study, median survival has been determined as 9,8 months. While partial response proportion has been found as 30%, stable disease proportion has been found as 40% (20). In another study where combination of Cisplatin with Pemetrexed and Gemcitabine were compared, median survival of 10,3 months has been obtained with Gemcitabine-Cisplatine (21). In the studies from Western countries, median survival varies between 9-11 months (22, 23). Ohen et al. have compared İrinotecan-Cisplatin combination with Paclitaxel-Carboplatin, Gemcitabine-Cisplatin and Vinorelbine-Cisplatin in Japan patients and have shown that these combinations had similar efficacy (24). In Gemcitabine-Cisplatin group of this study, median survival has been determined as 14 months and response percentage as 59.6%. Öztop et al. from our country has determined median survival as 13,2 months and response percentage as 65% (25). These response percentages are higher than Western studies. The reason for this may be ethnic differences.

Consequently, Gemcitabine-Cisplatin combination is an efficient treatment alternative with tolerable toxicity in advanced and metastatic phase NSCLC. In the number of chemotherapy cycles applied for response to this treatment, the differences in ethnicity can come into play besides gender. Determination of the factors which may impact this response will protect some patients from the complications of treatment and will prevent losing some patients due to treatment-related reasons.

References

1. *Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011; 61 (4): 212-236.*

2. *Non-small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. BMJ. 1995; 311: 899-909.*

3. *Kulesza P, Ramchandran K, Patel JD. Emerging concepts in the pathology and molecular biology of advanced non-small cell lung cancer. Am J Clin Pathol. 2011 ; 136(2): 228-238.*

4. *Ranson M, Davidson N, Nicolson M, et al. Randomized trial of paclitaxel plus supportive care versus supportive care for patients with advanced non-small-cell lung cancer. J Natl Cancer Inst. 2000; 92(13): 1074-1080.*

5. *Roszkowski K, Pluzanska A, Krzakowski M, et al. A multicenter randomized, phase III study of docetaxel plus best supportive care versus best supportive care in chemotherapy-naive patients with metastatic or non-resectable localized non-small cell lung cancer (NSCLC). Lung Cancer. 2000; 27(3): 145-157.*

6. *Depierre A, Lemarie E, Dabouis G, Garnier G, Jacoulet P, Dalphin JC. A phase II study of Navelbine*

(vinorelbine) in the treatment of non-small-cell lung cancer. Am J Clin Oncol. 1991; 14(2): 115-119.

7. *Schiller JH, Harrington D, Belani CP. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346: 92-98.*

8. *Kaplan R, Lacombe D, Verweij J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).*

9. *Hertel LW, Boder GB, Kroin JS. Evaluation of the antitumor activity of Gemcitabine (2',2'-difluoro-2'-deoxycytidine). Cancer Res. 1990; 50: 4417–4420.*

10. *Abbruzzese JL, Grunewald R, Weeks EA. A phase I clinical, plasma, and cellular pharmacology study of Gemcitabine. J Clin Oncol. 1991; 9: 491–498.*

11. *Pollera CF, Ceribelli A, Crecco M. Prolonged infusion Gemcitabine: a clinical phase I study at low-(300 mg/m2) and high-dose (875 mg/m2)levels. Invest New Drugs. 1997; 15: 115–121.*

12. *Anderson H, Lund B, Bach P. Single- agent activity of weekly Gemcitabine in advanced non small cell lung cancer: A phase II study. J Clin Oncol 1994; 12: 1821-1826.*

13. *Le Chevalier T, Gottfried M, Gatzemeier U, Shepherd F, Weynants P, Cottier B, Groen HJ, Rosso R, Mattson K, Cortes-Funes H, Tonato M, Burkes RL, Voi M, Ponzio A. A phase II multicenter study of gemcitabine in non small cell lung cancers. Bull Cancer 1997 Mar; 84(3): 282-288.*

14. *Abratt RP, Sandler A, Crino L. Combined Cisplatin and Gemcitabine for non-small cell lung cancer: influence of scheduling on toxicity and drug delivery. Semin Oncol. 1998; 25: 35–43.*

15. *Rinaldi M, Crino L, Scagliotti GV. A three-week schedule of Gemcitabine-Cisplatin in advanced non-small-cell lung cancer with two different Cisplatin dose levels: a phase II randomized trial. Ann Oncol. 2000; 11: 1295–300.*

16. *Huisman C, Giaccone G, van Groeningen CJ, Sutedja G, Postmus PE, Smit EF. Combination of Gemcitabine and Cisplatin for advanced non-small cell lung cancer: a phase II study with emphasis on scheduling. Lung Cancer 2001; 33: 267-275.*

17. *Wakelee HA, Wang W, Schiller JH, Langer CJ, Sandler AB, Belani CP, Johnson DH; for the Eastern Cooperative Oncology Group. Survival differences by sex for patients with advanced non-small cell lung cancer on Eastern Cooperative Oncology Group trial 1594. J Thorac Oncol. 2006; 1(5): 441-446.*

18. *Nordquist LT, Simon GR, Cantor A, Alberts WM, Bepler G. Improved survival in never-smokers vs current smokers with primary adenocarcinoma of the lung. Chest. 2004; 126: 347-351.*

19. *Hoang T, Dahlberg S, Johnson JSD. Does Histology Predict Survival of Advanced Non-Small Cell Lung Cancer (NSCLC) Treated with Standard Platin-Based Chemotherapy? S493, abstr PD6.4.1. J Thorac Oncol. 2009.*

20. *Scagliotti GV, De Marinis F, Rinaldi M, et al. Italian Lung Cancer Project. Phase III randomized trial comparing three platinum-based doublets in advanced non-small-cell lung cancer. J Clin Oncol. 2002; 20(21): 4285-4291.*

21. *Scagliotti GV, Parikh P, von Pawel J. Phase III study comparing Cisplatin plus Gemcitabine with Cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol. 2008; 26: 3543–3551.*

22. *Sandler AB, Nemunaitis J, Denham C. Phase III trial of gemcitab plus Cisplatin versus Cisplatin alone in patients with locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol. 2000; 18: 122–130.*

23. *Treat JA, Gonin R, Socinski MA, Edelman MJ, Catalano RB, Marinucci DM, Ansari R. A randomized, phase III multicenter trial of Gemcitabine in combination with carboplatin or paclitaxel versus paclitaxel plus carboplatin in patients with advanced or metastatic non-small-cell lung cancer. Ann Oncol. 2010; 21(3): 540-547.*

24. *Ohe Y, Ohashi Y, Kubota K, Tamura T, Nakagawa K, Negoro S, Nishiwaki Y, Saijo N, Ariyoshi Y, Fukuoka M. Randomized phase III study of Cisplatin plus irinotecan versus carboplatin plus paclitaxel, Cisplatin plus Gemcitabine, and Cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Ann Oncol. 2007; 18(2): 317-323.*

25. *Öztop İ, Yavuzşen T, Tekiş D. Efficacy of Cisplatin/ Gemcitabine Chemotherapy Regimen in Advanced Non-Small-Cell Lung Cancer. Toraks Journal 2002; 3(3): 273-278.*

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A practice of reducing recognition failure in patient identification before medication administration

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Abstract

Objective: To reduce the recognition failure in patient identification before medication administration, and ensure medication safety.

Methods: We investigated the status quo of patient identification before medication administration, analyzed the critical factors of recognition failure, and took appropriate rectification measures to optimize nursing procedures, and compare its effect with that before improvement.

Results: The incidence of recognition failure in patient identification before medication administration was decreased from 46.92 percent to 18.56 percent.

Conclusion: The optimization of nursing workflow based on the status quo and critical factors of recognition failure in patient identification can improve the effectiveness of nursing management and ensure medication safety.

Key words: Patient identification, nursing optimization, medication safefy.

Introduction

To improve the recognition accuracy of medical staff on patient identification may guarantee the operation conducted suitable for patients, and it is also conducive to effectively protecting the interests of patients and medical workers, ensuring medical safety, and reducing adverse medical events [1]. The current application of pocket PC of personal digital assistant (PDA) can achieve bar code identification of patients, so as to effectively guarantee the accuracy of patient identification [2]. However, for hospitals with relatively weak foundation for information development, the identification before medical operation relies solely on the normative artificial checking processes, which exists a certain volatility and uncertainty and safety loopholes [3]. In this study, in order to reduce recognition failure in patient identification before medication administration, we identified its critical factors through investigation and analysis on wards, and took appropriate rectification measures to optimize nursing procedures, and compare its effect with that before improvement, which achieved a satisfactory effect.

Materials and Methods

Identification of improvement projects

The results of quality inspection in our hospital between October and December 2011 showed that the performance of ward nurses in patient identification before medication administration was not up to standard, with a relatively high incidence of recognition failure. It was mainly reflected in not checking bedside card, calling instead of asking the name of patient, or even confirming identity only by bed number, which was likely to cause checking errors. Therefore, we determined to take the reduction of recognition failure in patient identification as the key project of improvement.

Establishment of project team

The wards related to this project were determined in accordance with the requirements of wards and characteristics of different modes of medication administration, including department of orthopedics, gynecology, general surgery, geriatrics and endocrinology. There were 16 members in the team in total, with the head nurse of ****Department as the leader. The team consisted of the head nurse and two key nursing staffs of each ward. The hospital president served as the project support member, and the director of Quality Improvement Department the project instructor to provide technical support for quality improvement. The key point of quality control was indentified to be the recognition failure in patient identification before medication administration in wards. As for the non-standard methods to confirm a patient's identity previously, recognition failure was defined as

any behavior that did not meet the requirements in the process of patient identification [4].

Data measurement

All team members were convened to conduct analysis on personnel, methods, equipment, materials, environment and other aspects through the Fishbone Diagram obtained by brainstorming method [5] to identify relevant factors that might affect the recognition of nurses in patient identification (Fig. 1). The frame flow chart was made according to the identification check process before improvement (Fig.2). According to Fig. 1 and Fig. 2, the identification process (Y) was divided to be three aspects: Y1 for checking the information on the patient's bedside card (wrist strap), Y2 for bed number, and Y3 for name asking.

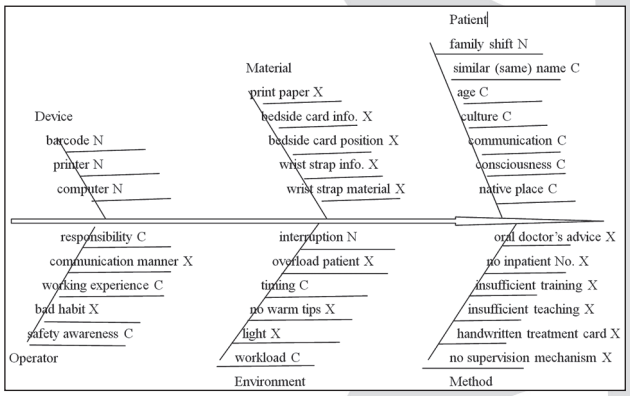

Figure 1. Fishbone diagram of identification-relating factors. X: controllable factor; C: uncontrollable factor; N: negligible factor.

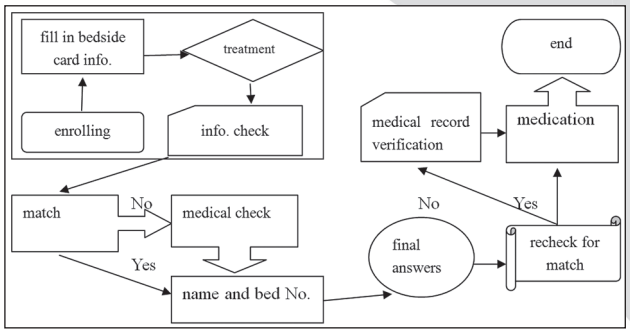

Figure 2. Identification check process before improvement.

The data collection form was specially designed, and the team members divided labor with individual responsibility, and assigned designated persons to track and acquire the administration operation of all nurses in each ward and perform on-site investigation on recognition failure in patient identification before medical operation and relevant impact factors respectively in the morning, at noon, in the afternoon and evening daily. In January 2012, a total of 521 cases of identification data in the five wards were collected for summary statistics. In order to ensure the authenticity of the data collection, all operational data were kept secret to all nurses in each ward except team members before the project analysis and improvement.

Data analysis

The Six Sigma special management tool-MiniTab software [6] was adopted to calculate the data collected in the measurement phase. The analysis results showed that the incidence of recognition failure in patient identification before medication administration in the five wards reached 46.92 percent, in which the failure numbers of Y1, Y2 and Y3 were 429,163 and 138 respectively, and the Z value of process capability was 1.621, indicating that there was considerable room for improvement.

The collected data showed that the incidence of recognition failure of Y1 for checking the information on patient's bedside card accounted for 82.34 percent, and that of the Geriatrics Department was 74.15 percent, which had a statistically significant difference compared with the other wards. In order to make up all the objective and subjective factors unable to be understood during the data tracking and acquisition, we conducted questionnaire survey on all the nurses and student nurses in the five wards and analysis on the data. Main survey contents included working years, post and other basic information of the respondents, their understanding of identification process and necessity, acceptable forms and times of training, objective and subjective factors influencing the check links of Y1, Y2 and Y3, the determination of bedside card information and recommendations for improvement. 80 copies of questionnaire were distributed in the five wards, and 72 copies returned.

According to the data of the above two surveys, significant factors affecting patient identification before medication administration by nurses were drawn by Pareto analysis [7] and Chi-square test as follows: X1 whether there is teaching training (affecting Y1, Y2 and Y3), X2 handwritten treatment card or lack of treatment card (affecting Y1

and Y3) , X3 whether there are warm tips (affecting Y1 and Y2) , X4 nurses or student nurses whose working time is within one year (affecting Y1 and Y2) , X5 position of bedside card is too low and card information is ambiguous (affecting Y1).

Establishment of project objectives

From an ideal prospective, the recognition of patient identification should ensure no fault at all. But it is a gradual process to develop a behavior habit [8]. According to the data measured on the basis of the status quo, the time for goal improvement was identified in June 2012. The incidence of recognition failure in patient identification before medication administration in each ward was decreased to 20 percent or less.

Improvement measures

Standardize teaching training

As for the impact factors of X1 and X4, we should organize special training regularly, list the knowledge related to identification system into the monthly vocational study plan, and conduct intensive training repeatedly for young nurses in the form of lecture and morning meeting quiz. We should organize typical case analysis, discussion and education, in order to eliminate the phenomenon of weak safety awareness of some nurses. New nurses and student nurses should be given with special routine teaching on their first day at work, which should be responsible by the intra-department head teacher.

Standardize nursing and administration operation

As for the impact factor X2, the operation procedures should be standardized, transfusion check card should be installed at the end of the bed in a unified manner, and all types of cards for nursing, administration and treatment are subject to computer printing. Hospitalization number is considered as the only effective identification of inpatients in management. It is necessary to give play to the identification function of hospitalization number and display the information of hospitalization number on the transfusion check card and medication treatment card. It is not allowed to only take bed number as the identification basis. We should implement the "three prohibitions" principle; that is, prohibit the use of handwritten treatment card, prohibit implementing doctor's verbal advice in non-emergency treatment, and prohibit all medication administrations without a treatment card. Head nurses and intra-department head teachers should strengthen link monitoring occasionally and pay special attention to operations of young nurses and student nurses.

Set up warm tips

As for the impact factor X3, warm tips should be set up in offices, treatment vehicles and bedside, etc. The tips should adopt anthropomorphic and colloquial phrases, with contents prominently straightforward. The special principal system of patient safety objective should be established, in which the person in charge is required to own the title of nurse practitioner or above with a working time more than eight years, and to be elected by secret ballot by the department staff. The special principal takes charge of conducting regular monitoring inspection and feed back tips every month.

Improve bedside card

As for the impact factor X5, we should collaborate with the computer center to modify the system software by use of existing information technology infrastructure, so that bedside card information can be printed after the admission of patients, which should highlight the two important information of patient name and hospitalization number with the 2nd boldface, and other information is printed with the 4th font. The information card should be inserted on the bedside where nurses can see the information clearly by standing with direct sight. The improved bedside card can avoid the shortcomings of fuzzy handwritten information, which is able to meet the current needs of nurses for check, no longer influenced by card position. From the point of view of cost saving, the improvement of bedside card position was not made in this project.

Specify standard and formulate continuous improvement measures

After identification flow chart before nursing and administration is improved, "fill in information on bedside card" and "check information on bedside card or wrist strap of patients" in the original procedures are respectively changed into

"print information on bedside card" and "take bottle label and treatment card for bilateral confirmation with the information on bedside card or wrist strap during the operation", as shown in the part with a box in Figure 2. On the basis of improvement implementation, the above improvement measures are regulated to be a system in the department. The special person in charge of safety goals should feed back the monitoring results in the department quarterly, and intra-department head nurse occasionally makes a sample inspection on the system implementation and responsibilities completion, so as to achieve continuous improvement. As the conditions of hospital information increasingly mature, our hospital will list the introduction of the PDA system into the short-term plan schedule so as to completely change the operation approach, and avoid artificial multi-procedure check and other unstable factors.

Results

The results summarized in Table 1 indicate that the improvement measures herein are effective with significant differences (P<0.01). All controllable factors after treatment were subjected to a multiple linear regression analysis.

Discussion

The incidence of recognition failure in patient identification in our hospital was decreased significantly through the improvement of the controllable factors affecting the failure, such as operators, methods, environment and materials, etc. It is thus clear that the improvement of the controllable factors play a great role in ensuring a correct patient identification [9], and to strengthen the management of controllable factors of recognition failure can guarantee the correct identification before nursing operation to the largest extent [10]. In this study, we should enhance the education of nursing staffs, pay attention to correct their bad habits and communication expressions, establish and improve the supervision and management mechanism [11], and strengthen care and medical training; in addition, as for the issue of patient identification failure due to missing or absence of materials, we should improve the ward bed responsibility system, and specially assign persons to take charge of material distribution and inspection; all the above measures can significantly reduce recognition failure in patient identification [12].

Correct patient identification before each nursing procedure is one of the most basic measures to ensure the safety of patients and prevent nursing

Table 1. Multiple linear regression analysis of controllable factors.

Controllable factor		Regression coefficient	Standard error	Normalized regression coefficient	t value	P value
Constant		15.117	2.793	—	5.413	<0.001
Operator	bad habit	-1.556	0.459	-0.291	-3.328	0.001
	communication manner	0.623	0.159	0.396	5.001	<0.001
Method	no supervision mechanism	0.567	0.109	0.781	6.249	<0.001
	handwritten treatment card	1.223	0.107	0.632	9.113	<0.001
	insufficient teaching	0.512	0.159	0.396	5.001	0.003
	no inpatient No.	0.391	0.205	0.732	5.321	<0.001
	insufficient training	-1.223	0.107	-0.430	-5.103	0.036
	oral doctor's advice	0.389	0.565	0.712	5.294	<0.001
Environment	no warm tips	1.563	0.237	0.801	7.256	<0.001
	light	-0.826	0.290	-0.567	-4.279	0.125
Material	wrist strap material	0.882	1.378	0.056	0.721	0.502
	wrist strap info.	3.201	1.371	0.195	2.413	0.017
	bedside card position	0.756	1.261	0.066	0.653	0.373
	bedside card info.	2.567	1.230	0.178	2.109	0.034
	print paper	2.057	0.518	0.293	3.682	0.005

$R=0.734$; $R^2=0.560$; $F=24.270$; $P<0.001$

errors [13]. Medical injuries suffered by hospitalized patients in the United States accounted for 3.5 percent, those caused by medication negligence or errors 7.0 percent [15]. Before in-depth investigation, we had thought that the main factors affecting a nurse's recognition of patient identification were some subjective factors, such as her sense of responsibility and safety awareness, in which it was difficult to put forward effective solutions before the introduction of the PDA information system [16]. As hospital administrators, we can not ignore hidden risks in medical safety and the occurrence of adverse events of medical safety resulting from it [17]. To exploit the existing resources available on the basis of the actual status of hospital and overcome subject and object of medical care are unstable factors in a pluralistic society[18]. To learn and apply various modern management tools and to propose practical quality improvement measures are effective methods for the improvement of the existing medical care work.

The management of all sections in hospital can not be completed and improved by department directors or front-line staff alone [19], which should be attached great importance and guided to by top hospital leaders [20] to introduce advanced management concepts [21], give full play to the wisdom of all staffs [22], and solve clinical problems in the form of project team, so as to effectively avoid "experience-dependent decision-making" [23]. Data is the result of measurement, and thus the data based on the fact is the basis of analysis and decision-making [24].

In this study, we investigated the status quo of patient identification before medication administration in five wards, collected data, and quantified quality fluctuation and instability so as to infer and identify key reasons for imperfect inspection by means of system analysis and data statistics and improve nursing procedures, so that all nurses can consciously perform patient identification before medical operation on a basis of the incomplete development of information system to guarantee medication safety of patients [25].

References

1. *Larsen G, Grant MJ. Pediatric patient safety: identification and characterization of adverse events, adverse drug events, and medical error. Pediatr Crit Care Med 2004; 5: 189-191.*

2. *Huang PJ, She CC, Chang P. The development of a Patient-Identification-Oriented Nursing Shift Exchange Support System using wireless RFID PDA techniques. AMIA Annu Symp Proc 2005: 990.*

3. *Bennett K, Bennett AJ, Griffiths KM. Security considerations for e-mental health interventions. J Med Internet Res 2010; 12: e61.*

4. *Zhao SM, Zhang SH, Li CT. [A primary study of criterion for source identification of gastrointestinal tumor sample with Identifiler multiplex system]. Fa Yi Xue Za Zhi 2011; 27: 102-106.*

5. *Pena A, Estrada CA, Soniat D, Taylor B, Burton M. Nominal group technique: a brainstorming tool for identifying areas to improve pain management in hospitalized patients. J Hosp Med 2012; 7: 416-420.*

6. *Shah D, Londhe V. Optimization and characterization of levamisole-loaded chitosan nanoparticles by ionic gelation method using 2(3) factorial design by Minitab 15. Ther Deliv 2011; 2: 171-179.*

7. *Teichert K, Suss P, Serna JI, Monz M, Kufer KH, Thieke C. Comparative analysis of Pareto surfaces in multi-criteria IMRT planning. Phys Med Biol 2011; 56: 3669-3684.*

8. *Henneman EA, Roche JP, Fisher DL, Cunningham H, Reilly CA, Nathanson BH, et al. Error identification and recovery by student nurses using human patient simulation: opportunity to improve patient safety. Appl Nurs Res 2010; 23: 11-21.*

9. *Nellans K, Schmalzried TP, Macaulay W. Effect of patient-controllable factors on survivorship of primary total hip arthroplasty. J South Orthop Assoc 2003; 12: 72-74.*

10. *Cavalleri M, Morstabilini R, Reni G. A wearable device for a fully automated in-hospital staff and patient identification. Conf Proc IEEE Eng Med Biol Soc 2004; 5: 3278-3281.*

11. *Cheng C, Li LM, Alves P, Gerstein M. Systematic identification of transcription factors associated with patient survival in cancers. BMC Genomics 2009; 10: 225.*

12. *Marquard JL, Henneman PL, He Z, Jo J, Fisher DL, Henneman EA. Nurses' behaviors and visual*

scanning patterns may reduce patient identification errors. J Exp Psychol Appl 2011; 17: 247-256.

13. *Phipps E, Turkel M, Mackenzie ER, Urrea C. He thought the "lady in the door" was the "lady in the window": a qualitative study of patient identification practices. Jt Comm J Qual Patient Saf 2012; 38: 127-134.*

14. *Mion LC, Chandler AM, Waters TM, Dietrich MS, Kessler LA, Miller ST, et al. Is it possible to identify risks for injurious falls in hospitalized patients? Jt Comm J Qual Patient Saf 2012; 38: 408-413.*

15. *Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 2005; 16: 3365-3370.*

16. *Lippi G, Plebani M. Identification errors in the blood transfusion laboratory: a still relevant issue for patient safety. Transfus Apher Sci 2011; 44: 231-233.*

17. *Atinga RA, Mensah SA, Asenso-Boadi F, Adjei FX. Migrating from user fees to social health insurance: exploring the prospects and challenges for hospital management. BMC Health Serv Res 2012; 12: 174.*

18. *Leontowitsch M, Higgs P, Stevenson F, Rees Jones I. Review: Taking care of yourself in later life: a qualitative study into the use of non-prescription medicines by people aged 60+. Health (London) 2010; 14: 213-231.*

19. *O'Sullivan TL, Dow D, Turner MC, Lemyre L, Corneil W, Krewski D, et al. Disaster and emergency management: Canadian nurses' perceptions of preparedness on hospital front lines. Prehosp Disaster Med 2008; 23: s11-18.*

20. *Morin D, Aubin M, Vezina L, Gagnon J, Racine S, Reinharz D, et al. From hospital to home after cardiac surgery: evaluation of a community nursing care management model. Prof Case Manag 2009; 14: 167-175; quiz 176-167.*

21. *Beddock R, Naepels P, Gondry C, Besserve P, Camier B, Boulanger JC, et al. [Diagnosis and current concepts of management of advanced abdominal pregnancy]. Gynecol Obstet Fertil 2004; 32: 55-61.*

22. *Wu WX,Zheng XY,Xie HY,et al. [Shortened practice and effect of patients discharged from hospital waiting time]Chinese Journal of Nursing 2010; 45(10): 925-926.*

23. *Miao T, Zhu JH, Wang SJ, et al.[Six Sigma management to reduce the practice of outpatient intravenous infusion drip rate defects] Chinese hospitals 2010; 14 (6): 63-65.*

24. *Rittmeister M, Kerschbaumer OF. [Medications in orthopedics. Data, facts, evidence-based statements]. Orthopade 2003; 32: 1069.*

25. *Creus Baro N, Masso Muniesa J, Codina Jane C, Ribas Sala J. [Cytostatic drug identification using bar codes: an essential need for patient safety]. Farm Hosp 2005; 29: 214.*

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The effects of caffeic acid phenethyl ester on rat random patern skin flaps under the influence of nicotine

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Abstract

Background: The negative effects of cigaratte on flap survive have been demonstrated in various experimental and clinical researches. The aim of this study is to research of the effects of caffeic acid phenethyl ester (CAPE) on the increase of random patern skin flap necrosis under the influence of nicotine.

Materials and Methods: In the study, 40 rats were divided into 8 groups. The first group, as the control group, received only serum physiologic (SF) throughout the experiment. For four weeks, nicotine was subcutaneously given as 2 mg/kg dose to the other four groups. At the end of the 4th week, the 3x9 cm caudal based random patern flaps at the dorsum of the rats were removed and resutured. During the postoperative period, for seven days, starting from the 2nd group SF, nicotine, CAPE and nicotine+CAPE were given to the groups, respectively.

Results: The average rates of flap necrosis in the 5 groups were determined, respectively, as 21,0 ± 3,5 %, 35,2 ± 6,0 %, 39,3 ± 7,1 %, 23,2 ± 3,3 %, and 27,0 ± 5,0 %. Significant differences were identified in the necrosis percentages between groups 1-2, groups 1-3, groups 2-4, groups 3-4, and groups 3-5 (p = 0.001). In histopathologic evaluation, the amount of PMNL was determined to be low in the control group and the 4th and 5th groups receiving postoperative CAPE, and high in the other groups.The amount of lymphocyte was found out to be high in the 3rd group receiving both pre-operative and postoperative nicotine, quite low in the control group, moderate and similar in the other groups. Vascular proliferation, edema, fibroblast and the amount of collagen were identified to be high in 2nd and 3rd groups which did not receive postoperative treatment, low and similar in the other groups. MDA values, compared to the other groups, were found out to be low in the control group and the groups given CAPE (p = 0.001).

Conclusion: The results of this study, the results of the flap surface area measurement along with the histopathologic and biochemical evaluations indicate that adverse effects on random pattern skin flaps caused by nicotine can be reduced with the use of CAPE.

Key words: Skin flap, flap survive, nicotine, caffeic acid phenethyl ester, rat.

Introduction

Random pattern skin flaps are used very often in Plastic and Reconstructive surgery. In order to avoid complications in flap surgery, correct selection and planning of flap and correct surgical technique are essential, however, not enough [1]. Systemic factors related to the patient also affect the flap survive. Smoking and nicotine indirectly, is one of these factors. The effect of nicotine on the flap depends on the dose and the time [2]. Nicotine performs these negative effects through a variety of mechanisms. Harmful effects of nicotine on the skin vascular structures were explained by mechanisms such as doing direct endothelial damage by creating free oxygen radicals, and causing vasoconstriction by increasing the release of norepinephrine from sympathetic nerve endings and by stimulating the release of catecholamines from the adrenal glands [3].

In order to reverse the harmful effects of smoking on the skin various pharmacological agents were tried. Agents such as Calcium channel blockers,

which cause peripheral vasodilation [3], pentoxifylline as a rheological agent [4], phenoxybenzamine, nitroglycerin [5] and α_1 receptor blocker terazosin [6] are some of the pharmacological agents studied since they were thought to reduce flap necrosis caused by nicotine. Due to lack of research about the effect of CAPE, a powerful antioxidant and anti-inflammatory agent, on the flap survive under the influence of nicotine in the examination of literature, our study was planned taking into account the effects of nicotine on oxidative stress and the effects increasing free radical formation.

Materials and methods

This experimental study was carried out in Pamukkale University Faculty of Medicine Experimental Animal Research Laboratory after the approval by the Board of Ethics. 40 Wistar albino rats whose weights ranged from 204-242 gm were used. All rats were fed with standard rat food and tap water. They were kept in rooms with a light pattern which is 12 hours light and 12 hours dark and with a temperature of about 21 °C.

Surgical Method

Anesthesia was performed with the injection of IM 90mg/kg a ketamine-HCl and 10mg/kg Xylasine-HCL on the experimental animal. After following the depth of anesthesia with jaw and skeletal muscle tonus, the hairs on the backs of the rats were shaved in the prone position on the surgical table. Later, McFarlane-type [7] dorsal skin flap about 3x9 cm in size on the caudal pedicle was planned and drawn with the drawing pen (Figure 1A). After the incision of flap edges with a scalpel of number 15, random pattern skin flaps were removed by blunt dissection to include the skin and panniculus carnosus. Flap 5/0 was sutured to the bed continuously with atraumatic silk (Figure 1B). The rules of asepsis were followed during the procedures.

Figure 1. Planning and drawing (A) returning and suturing the flap (B).

Experimental Protocol and Groups

The subjects were divided into 5 groups of 8 rats. 2nd, 3rd, 4th and 5th groups received a daily dose of 2 mg / kg dose of nicotine, 28 days, subcutaneously (sc). The first group was injected 0.3 ml of saline (SF) sc, in this period. At the end of the 28th day, anesthesia was established by IM 90mg/kg ketamine-HCL and 10mg/kg HCL Xylasine, and surgery was performed in all groups.

In the postoperative period, for seven days 1st and 2nd groups were given 0.3 ml sc and 0.5 ml intraperitoneal (ip); 3rd group received 2 mg / kg dose of nicotine sc and 0.5 ml ip SF; 4th Group was given 10 micromole /kg dose of CAPE ip and 0.3 ml sc; 5th group was given 2 mg / kg dose of nicotine sc and 10 micromole /kg dose of CAPE ip (Table 1).

At the end of the seventh postoperative day, under general anesthesia, first the flap necrosis and the living area of all subjects were measured. Intracardiac blood samples were taken from the subjects. Then, about 1 cm^2 from each, tissue biopsy specimens were obtained from the live transitional zone of flap-necrosis area of skin for the histopathologic and biochemical evaluation.

Table 1. Summary of experimental design

	Preop. 28 days		Postop. 1-7 days
Group 1	SF	Surgery	SF
Group 2	Nicotine		SF
Group 3	Nicotine		Nicotine
Group 4	Nicotine		CAPE
Group 5	Nicotine		Nicotine - CAPE

Evaluations

For clinical evaluation, within a 3x9 cm flap area, the ratio of necrotic area to all flap area, and for histopathologic evaluation, six parameters which are the amount of PMNL, lymphocyte density, capillary proliferation, fibroblast proliferation, collagen density and edema were taken into consideration. In biochemical evaluation, malondialdehyde levels (MDA) were measured in serum and tissue.

Surface Area Evaluation

Conditions of flaps were observed daily.At the end of the 7th postoperative day, the flaps of anesthetized subjects were photographed in equal distance with the digital camera, Canon IXUS 70 (Canon Inc. JAPAN). Photos were plotted on a graph using the Photoshop CS4 (Adobe Systems. USA) and Windows 7 (Microsoft Corporation. USA) programs. In Photoshop program, first by drawing the necrotic area and then all the flap, the pixel numbers were recorded. Values were proportioned to each other, and expressed as percent. The areas considered to be suspicious in flaps were evaluated in terms of necrocis.

Histopathologic Evaluation

Histopathologic evaluation was carried out by a pathologist who did not know which group the specimen examined belonged to. 1x1 cm tissue biopsies of the groups taken from the transitional zone between flap necrotic and intact tissue on the seventh day, after being identified in 10 % formalin, were integrated into paraffin blocks. 4-micron thick sections were obtained by microtome. Covered with hematoxylin and eosin, sections were examined under a light microscope. In the examples six parameters which are PMNL, the amount of lymphocyte, capillary vascular proliferation, edema, fibroblast proliferation and the amount of collagen were considered.

Biochemical Evaluation

Thiobarbituric acid and absorbance measured at 532 nm in an acidic environment malondialdehyde principle of measurement is based on the color formed by Ohkawa et al. were measured by applying the method [8]. The amount of MDA in the specimen was calculated based on the calibration graph drawn using the standard 1,1,3,3-tetraetoksipropan, and was expressed as nmol /g tissue. MDA serum level was measured by working on serum with the same procedures, and was expressed as nmol / g.

Statistical Analysis

For statistical analysis, defining Kruskal-Wallis Variance Analysis and tests and Bonferroni corrected Mann-Whitney-U tests were used. The SPSS program was used in the analysis of all statistical tests (SPSS 10.0 for Windows; Chicago, IL, USA). Regarding statistical significance, a p value of < 0.05 was considered significant.

Results

Surface Area Assessment Results

In this study, investigating the effect of CAPE on skin flaps under the influence of nicotine, the mean necrosis rates in flaps were determined as 21.0 ± 3.5 % in group 1, 35.2 ± 6.0 % in group 2, 39.3 ± 7.1 % in group 3, 23.2 ± 3.3 % in group 4, and 27.0 ± 5.0 % in group 5, respectively (Figure 2). Each group was statistically compared with one another. Statistically significant differences were found in the percentage of necrosis between groups 1 - 2 ($p = 0.001$), groups 1 - 3 ($p = 0.001$), groups 2 - 4 ($p = 0.001$), groups 3 - 4 ($p = 0.001$) and groups 3 - 5 ($p = 0.001$).

According to these results, the rate of necrosis was found out to increase in the group receiving preoperative nicotine compared to the control group. The highest rate of necrosis was found in the group receiving both preoperative and postoperative nicotine. The rate of necrosis was determined to be lower in the group continuing to receive postoperative nicotine and given CAPE than in the 2nd and 3rd groups which were not given CAPE. The group with the lowest rate of necrosis within the treatment groups was found out to be the group given only postoperative CAPE (Group 4). Although the rate of necrosis in this group was higher than the control group, it was not statistically significant ($p = 0.328$).

Histopathologic Evaluation Results

On the 7th postoperative day, PMNLs, lymphocytes, vascular proliferation, edema, fibroblast and the amount of collagen were evaluated in the tissue samples taken from the transitional zone of flap necrosis-normal tissue. Results were scored as

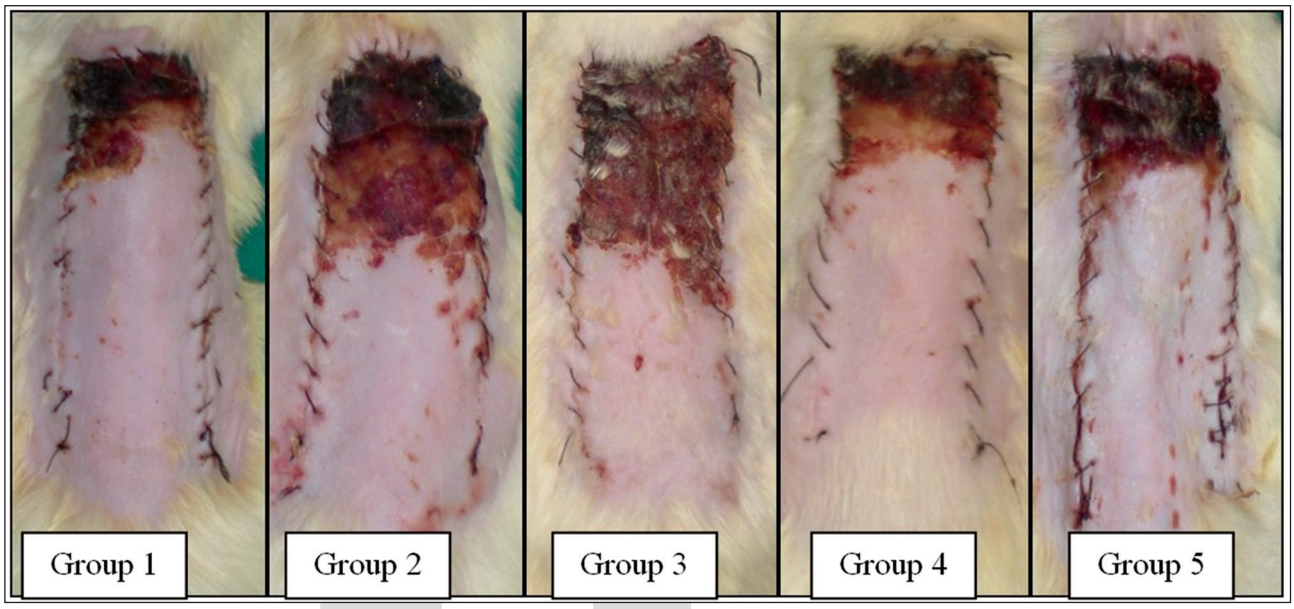

Figure 2. Sample photos taken of the control and the experimental groups on postoperative 7th day

low, medium and high between 1 and 3, and were compared with each other statistically (Table 2).

There was statistical significance among the groups in terms of the amount of PMNL (p=0.0001). Differences were identified between groups 1 - 2 (p=0,002), groups 2 - 4 (p=0,001), groups 2 - 5 (p=0,002) and groups 3 - 4 (p=0,001). In scoring values of the amount of lymphocyte, significant difference was found only between groups 1 - 3 (p=0.044). In scoring values of the proliferation of blood vessels, there was significant difference among the groups (p=0.0001). Statistically significant differences were identified between the groups 1-2 (p = 0.002), groups 1 - 3 (p = 0.001), groups 2 - 4 (p = 0.002), groups 2 - 5 (p = 0.002), groups 3 - 4 (p = 0.001), and groups 3 - 5 (p = 0.001). In terms of edema, there was statistical significance in scoring values (p = 0.0001). Statistically significant differences were found between the groups 1 - 2 (p = 0.002), groups 2 - 4 (p = 0.001) and groups 2 - 5 (p = 0.001). Significant differences were observed also in scoring values of the amount of fibroblasts (p = 0.0001). Statistically significant differences were found between the groups 1 - 2 (p = 0.002), groups 1 - 3 (p = 0.002), groups 2 - 4 (p = 0.001), groups 2 - 5 (p = 0.001) and groups 3 - 5 (p = 0.001). Finally, significant differences were identified in scoring values in terms of the amount of collagen (p = 0.0001). These differences were found between the groups 1 - 2 (p = 0.001), groups 1 - 3 (p = 0.002), groups 2 - 4 (p = 0.001), groups 2 - 5 (p = 0.001), groups 3 - 4 (p = 0.001) and groups 3 - 5 (p = 0.001).

According to the results, the amount of PMNL was low the control group and the 4th and the 5th groups receiving postoperative CAPE, and high in the other groups. The amount of lymphocyte was found out to be high in the 3rd group given both preoperative and postoperative nicotine, very low in the control group, and moderate and similar in the other groups, respectively. Vascular proliferation, edema, fibroblast and the amount of collagen were determined to be high in the 2nd and 3rd groups which did not receive postoperative treatment, and low and similar in the other groups, respectively.

Table 2. Mean scores of histopathologic examination and standard deviations of the groups

	Group 1	Group 2	Group 3	Group 4	Group 5
PMNL	1.25 ±0.46	2.75 ±0.46	2.68 ±0.74	1.13 ±0.35	1.25 ±0.46
Lymphocytes	1.38 ±0.52	1.75 ±0.46	2.25 ±0.46	1.75 ±0.71	1.50 ±0.53
Vascular proliferation	1.50 ± 0.53	2.88 ± 0.35	3.0 ± 0	1.63 ± 0.52	1.13 ±0.35
Edema	1.50 ±0.53	2.63 ±0.52	2.13 ±0.64	1.25 ±0.46	1.25 ±0.46
Fibroblast	1.25 ±0.46	2.75 ±0.52	2.88 ±0.35	1.63 ±0.52	1.25 ±0.46
Collagen	1.13 ±0.35	2.63 ±0.52	2.50 ±0.53	1.13 ±0.35	1.13 ±0.35

Biochemical Evaluation Results

On the 7th postoperative day, MDA, as an indicator of lipid peroxidation, were studied in serum and tissue samples taken from the transitional zone of flap necrosis-normal tissue. At the end of the study, results of all subjects were given the in Table 3.

Table 3. Serum and tissue MDA levels in control and experimental groups

	Serum MDA nmol/ml	Tissue MDA nmol/gr
Group 1	8.6 ± 1.1	207.8 ± 14.4
Group 2	11.8 ± 3.4	302.8 ± 38.6
Group 3	14.1 ± 1.4	350.1 ± 32.9
Group 4	8.8 ± 1.2	151.0 ± 22.2
Group 5	9.5 ± 0.8	204.2 ± 14.6

Statistically significant differences were identified in serum MDA levels between the groups 1 - 2 (p = 0.001), groups 1 - 3 (p = 0.001), groups 2 - 4 (p = 0.002), groups 2 - 5 (p = 0.003), groups 3 - 4 (p = 0.001) and groups 3 - 5 (p = 0.001). Statistically significant differences were found in scoring values in terms of the results of MDA between the groups 1 - 2 (p = 0.001), groups 1 - 3 (p = 0.001), groups 1 - 4 (p = 0.001), groups 2 - 4 (p = 0.001), groups 2 - 5 (p = 0.001), groups 3 - 4 (p = 0.001), groups 3 - 5 (p = 0.001) and groups 4 - 5 (p = 0.001). According to these results, MDA, an indicator of lipid peroxidation in serum, in other words, tissue damage, was determined to be high in the 2nd and 3rd groups, being the highest in the group receiving postoperative nicotine whereas it was low in treatment groups (groups 4 and 5) and in the control group. MDA levels of the group receiving postoperative CAPE nicotine for four weeks were found to be very close to the control group. Tissue MDA levels were determined to be parallel to serum levels with the only difference being that MDA level was lower in the group given postoperative CAPE than in the control group.

Discussion

The results of this study, along with the results of flap surface area measurement, histopathological and biochemical evaluations show that the adverse effects of nicotine on random pattern skin flaps can be reduced with the use of CAPE.

According to the results of flap surface area measurement, the rate of necrosis is higher in the group receiving preoperative nicotine, compared to the control group. In the case of continuous postoperative nicotine, the rate of necrosis continues to increase more. Taking preoperative nicotine and receiving postoperative CAPE treatment results in a statistically significant decrease in the rate of necrosis. Similarly, CAPE treatment reduced the rate of necrosis in the group taking preoperative nicotine and continuing postoperative nicotine.

In histopathological evaluation, while PMNL and lymphocyte infiltration and tissue edema were identified to be at normal levels in the control group and in the groups treated with CAPE, they increased significantly in nicotine groups. Capillary proliferation and the amount of fibroblast and collagen in tissue were high in nicotine group whereas they were at normal levels in control group and in the group receiving CAPE treatment. CAPE also biochemically reduced the negative effects of nicotine. In both serum and tissue, MDA levels, an indicator of lipid peroxidation, were lower in the control and CAPE groups, where as they were higher in nicotine groups.

As a result, CAPE, with its antioxidant and anti-inflammatory effect, may prevent the increase of necrosis caused by nicotine on rat random pattern skin flap.

Rohrich et al. listed ideal properties of a medicine that may be used to improve the liveability of the flap or an application as practical, reliable, inexpensive, and as having a clear mechanism [9]. Many agents have been studied to find this ideal medicine.

In the literature, there are studies on experimental flap models designed to reduce necrosis random pattern skin flaps under the influence of nicotine, vascular endothelial growth factor (VEGF), ketorolac, topical lidocaine and prilocaine, dexamethasone or carnitine, pharmacological agents such as topical oleic acid or nitric oxide, as well as sympatolytics, free radical sweepers, hemorheological agents, vasodilators and hyperbaric oxygen [3-6].

Rinker et al. studied the effect of calcium channel blockers in the rats exposed to cigarette smoke [3]. In this study, nifedipine and verapamil given enterally were shown to decrease the rate of necrosis in random pattern rat dorsal skin flap, significantly.

Aker et al. used pentoxifylline, a rheological agent to prevent flap necrosis in random pattern rat dorsal skin flap under the influence of nicotine, preoperatively [4]. It was indicated that preoperative pentoxifylline treatment, particularly for the patients who cannot give up smoking, may be useful in reducing skin flap necrosis.

Davies et al. used phenoxybenzamine and nifedipine enterally, and nitroglycerin topically in their studies, and reported that phenoxybenzamine treatment did not show a significant effect whereas with nitroglycerine and nifedipine there was a significant decrease in the necrosis [5].

Karlen et al. used terazosin, α_1 adrenoceptor antagonist, in the flaps under the influence of nicotine orally and subcutaneously, and reported increase in oral terazosin and survival of flap [6].

Selcuk et al. presented the positive effects of hyperbaric oxygen therapy on rat random pattern flap survival, in skin flaps under the influence of nicotine [10].

In another study, Baykan et al. reported that angiotensin 1-7 triggered angiogenesis by increasing vasodilation in nicotinized flaps and increased flap survival [11].

In the examination of the literature, no research was found about the effect of CAPE, which is an antioxidant agent, on flap survival under the influence of nicotine. In several experimental studies, CAPE was shown to have strong antioxidant effects [12-15]. MDA is the end product of lipid peroxidation and is used as an indicator of tissue damage. Through a lot of research in the literature, CAPE has been proven to reduce tissue MDA levels significantly [16-18].

Aydogan et al. studied the effects of CAPE on ischemia-reperfusion injury in rat random pattern skin flap. As a result, CAPE was reported to reduce flap necrosis rates and MDA levels significantly [16].

As a result, in the light of the data we have collected in our study, we can say that CAPE, antioxidant and anti-inflammatory substance, has positive effects on the increase in flap necrosis caused by the effect of nicotine. However, considering that nicotine along with more than one mechanisms have adverse effects on flap survival, combination of CAPE with other pharmacological agents may provide better results.

Limitations

In studies researching necrosis developing in the flaps after nicotine treatment, it was stated that there was no difference between the impacts of providing long-term low-dose nicotine (0.6 mg/kg/24 weeks) and giving short-term high-dose nicotine (2mg/kg/4 weeks) [19]. In our study, according to this information and due to the convenience of application, the method of short-term high-dose nicotine was preferred.

In addition, only the postoperative effects of CAPE were investigated in our study. When this model is adapted to the clinic, it addresses to smoking patients, emergency cases or flap surgery attempts carried out when smoking cannot be quitted. Considering the effects of CAPE, it may reduce the negative effects of smoking when smoking addict take it in preoperative period. Further experimental studies will be informative on this subject.

References

1. *Nezami BG, Rahimpour S, Sadeghi M, Sianati S, Anaraki DK, Ebrahimi F, Ghasemi M, Dehpour AR. Chronic lithium impairs skin tolerance to ischemia in random-pattern skin flap of rats. J Surg Res. 2011; 171: 374-8.*

2. *Forrest CR, Pang CY, Lindsay WK. Dose and time effects of nicotine treatment on thecapillary blood flow and viability of random pattern skin flaps in the rat. Br J Plast Surg 1987; 40: 295-9.*

3. *Rinker B, Fink BF, Barry NG, Fife JA, Milan ME. The effect of calcium channel blockers on smoking-induced skin flap necrosis. Plast Reconstr Surg 2010; 125: 866-71.*

4. *Aker JS, Mancoll J, Lewis B, Colen LB. The effect of pentoxifylline on randompattern skin flap necrosis induced by nicotine treatment in the rat. Plast Reconst Surg 1997; 100: 66-71.*

5. *Davies BW, Lewis RD, Pennington G. The impact of vasodilators on random patternskin flap survival in the rat following mainstream smoke exposure. Ann Plast Surg1998; 40: 630-6.*

6. *Karlen, RG, Maisel RH. Terazosin blockade of nicotine-induced skin flap necrosis in the rat. Arch Otolaryngol Head Neck Surg 1997; 123: 837-840.*

7. *McFarlane RM, DeYoung G, Hehry RA, et al. The design of a pedicle flap in the rat tostudy necrosis and its prevention. Plast Reconstr Surg. 1965; 35: 177-82.*

8. *Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8.*

9. *Rohrich RJ, Cherry GW, Spira M. Enhancement of skin-flap survival using nitroglycerin ointment. Plast Reconstr Surg 1984; 73: 943-8.*

10. *Selcuk CT, Kuvat SV, Bozkurt M, Yasar Z, Gulsun N, Ilgezdi S, Ula M, Burhan Ozalp B. The effect of hyperbaric oxygen therapy on the survival of random pattern skin flaps in nicotine-treated rats. J Plast Reconstr Aesthet Surg 2012; 65, 489-493*

11. *Baykan H, Gunay GK, MD, Ozyazgan I, Soyuer I. The Effect of Angiotensin (1–7) onSurvival of Random Pattern Skin Flaps With Nicotine-Induced Ischemia in Rats. Ann Plast Surg 2012; 68: 88–93*

12. *Russo A, Longo R, Vanella A. Antioxidant activity of propolis: role of caffeic acidphenethyl ester and galangin. Fitoterapia 2002; 73: 21-9.*

13. *Ilhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H, Akyol O. The effects of caffeic acidphenethyl ester (CAPE) on spinal cord ischemia / reperfusion injury in rabbits. Eur J Cardiothorac Surg 1999; 16: 458-63.*

14. *Ozyurt B, Gulec M, Ozyurt H, Ekici F, Atis O, Akbas A. The effect of antioxidant caffeic acid phenethyl ester (CAPE) on some enzyme activities in cisplatin-induced neurotoxicity in rats. Eur J Gen Med 2006; 3: 167-172.*

15. *Rossi A, Longo R, Russo A, Borrelli F, Sautebin L. The role of the phenethyl esterof caffeic acid (CAPE) in the inhibition of rat lung cyclooxygenase activity by propolis. Fitoterapia 2002; 73: 30-7.*

16. *Aydogan H, Gurlek A, Parlakpinar H, Askar I, Bay-Karabulut A, Aydogan N, et al.Beneficial effects of caffeic acid phenethyl ester (CAPE) on the ischaemia-reperfusion injury in rat skin flaps. J Plast Reconstr Aesthet Surg 2007; 60: 563-8.*

17. *Hosnuter M, Gurel A, Babuccu O, Armutcu F, Kargi E, Isikdemir A. The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury. Burns 2004; 30: 121-5.*

18. *Ozyurt H, Ozyurt B, Koca K, Ozgocmen S. Caffeic acid phenethyl ester (CAPE) protects rat skeletal muscle against ischemia-reperfusion-induced oxidative stres Vascul Pharmacol 2007; 47: 108-12.*

19. *Forrest CR, Pang CY, Lindsay WK. Pathogenesis of ischemic necrosis in random-pattern skin flaps induced by long-term low-dose nicotine treatment in the rat. Plast Reconstr Surg 1991; 87: 518-28.*

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Insertion/deletion polymorphism of the angiotensin converting enzyme gene in coronary artery disease in Iran

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Abstract

The role of angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism in coronary artery disease is controversial.

Here we examine the association between I/D polymorphism of the ACE gene and the presence of CAD in diabetic patients, who have the highest rates of CAD in Iran. The ACE (I/D) genotypes were detected by PCR in 510 individuals undergoing their first coronary angiography. All patients that had type 2 diabetic mellitus were placed into two groups: with CAD and without CAD.

Our results showed a significant correlation between the possession of the ACE D allele and CAD in patients (OR=1.55, p=0.001; $\chi 2$=9.5, p=.002). In conclusion,the I/D polymorphism of ACE gene (carrying D allele) is an independent risk factor for CAD in the studied Iran population. Larger collaborative studies are needed to confirm these results.

Key words: Angiotensin converting enzyme, coronary artery disease, diabetes mellitus, genetic polymorphism.

Introduction

In developing countries such as Iran coronary artery disease (CAD), type 2 diabetes mellitus (T2DM) and stroke is a major cause of death and disability. CAD accounts for nearly 50% of all deaths per year in Iranian population[1–7]. As known, Type 2 diabetes mellitus (T2DM) and CAD are multifactorial diseases influenced by environmental and genetic factors[8, 9]. The major complications of T2DM include nephropathy, neuropathy, retinopathy, and coronary artery diseases[10, 11]. Various genetic polymorphisms such as angiotensin converting enzyme (ACE) gene polymorphism were implicated as possible pathogenetic factors in CAD[12]. Angiotensin converting enzyme (ACE) gene is located on chromosome 17q23 and consists of 26 exons and 25 introns. ACE polymorphism is due to the presence (insertion (I) allele) or absence (deletion (D) allele) of a 287 bp Alu repeat sequence within intron 16 resulting in three genotypes DD and II homozygotes, and ID heterozygotes[13,14]. This polymorphism leads to three genotypes: DD, ID and II. While the positive correlation between DD genotype and cardiovascular disorders has been well studied[15], there are conflicting reports on association of the ID genotype with increased susceptibility to CAD[15, 16, 17]. Many investigators have followed the same line of inquiry, studying the relationship between this polymorphism and cardiovascular disease, although there are different reports in some, there is no positive significant relationship between ACE I/D polymorphism and cardiovascular disease [18]. In the present study, We investigated ACE genotype and allele distribution in patients with CAD in the diabetic Iranian population.

Methods

Study subjects

This is a case–control study. Patients recruited in this study (n = 510; 141 T2D with CAD and 369 T2DM without CAD) were angiographically identified as CAD in Imam Hospital of Tehran Univer-

sity of Medical Sciences, and then a detailed medical history of each patient was obtained. All patients were interviewed and data on smoking habit, body mass index (BMI; according to Quetelet equation by using the following formula: BMI= weight in kilograms/height in meters squared), blood pressure, lipid profile and family history of CAD were recorded. The diagnosis of diabetes was based on criteria from the American Diabetes Association[19].

Chemical analysis

After a 12 h overnight fasting, serum TC, TG, LDL-C and HDL-C levels were measured by standard enzymatic methods**.** Very-low-density lipoproteins (VLDLs) were calculated by Friedewald's formula[20].

DNA analyses

Five milliliters of peripheral blood samples was collected in tubes containing ethylenediaminetetraacetic acid. Genomic DNA was isolated from peripheral blood leukocytes by standard methods and stored at –20ºC. Genotyping of the ACE I/D polymorphisms in the ACE gene were determined by employing the polymerase chain reaction method[21].

Statistical analysis

The SPSS statistical software package version 16 was used for the statistical analyses. A p value of 0.05 or less was considered significant. The gene counting method calculated allelic frequencies. The $\chi 2$-test was used to verify the agreement of observed genotype frequencies with those expected according to the Hardy–Weinberg equilibrium. The genotypes and alleles frequencies of ACE were compared between T2DM patients with and without CAD using $\chi 2$- test. Odds ratios (OR) were calculated as estimates of relative risk for disease and 95% confidence intervals (CI) obtained by SPSS logistic regression.

Results

The clinical and laboratory features of the subjects found in CAD patients and in the control groups (patients without CAD) are reported in Table 1. Among the 510 patients with type 2 diabetes, 141 patients had CAD and 369 patients were without CAD. The mean age distribution in CAD patients was slightly higher than that of the control group (patients without CAD) but not significant. In addition, Cholesterol, TG, and LDL concentrations were significantly higher, in the CAD patients than in patients without CAD. The genotypic distribution among subjects was tested for Hardy-Weinberg equilibrium using the Chi-square test. The frequency of genotypes did not deviate from the Hardy-Weinberg equilibrium in total sample and in 2 study groups ($P > 0.05$). Distribution of ACE genotypes and allele frequencies in CAD and in the control subjects (patients without CAD) are presented in Table 2.

Distribution of ACE genotypes and alleles ($\chi 2=9.387$, $p=.009$; $\chi 2=9.5$, $p=.002$), except I/D genotype, were significantly different in CAD compared with patients without CAD. Gene counting was done for calculation of gene alleles by the Fisher exact test and manual counting.

The odds ratio (OR) for CAD patients, their ACE genotypes, and the copy numbers of ACE D allele with 95% confidence intervals are shown in Table 3. The ORs for the presence ACE (I/D+D/D) genotype and D allele in the CAD patients were found to be 1.98 (1.18- 3.3, $p=0.01$) and 1.55 (1.2-2.1, $p=0.001$), respectively.

These data suggest that the individuals with the presence of ACE-D allele increase the risk of CAD in diabetic patients in population of Iran.

Discussion

Coronary artery disease (CAD) is a multifactorial disorder with genotype and environmental interactions having an important role in its development. Therefore in addition to established risk factors, genetic risk factors may have important roles in the pathogenesis of coronary atherosclerosis and acute myocardial infarction[22]. The results of this study clearly demonstrate that the ACE D allele is an independent risk factor for CAD from population living in Iran. The distribution of the ACE I/D polymorphism in CAD patients ($\chi 2=.9387$, $df=2$, $p=0.009$) were found to be significantly different from that of the control group. The frequency of the ACE D allele was found to be significantly higher in CAD patients than in the control group. The percentage frequency of ACE D in CAD subjects were 60.6% compared to

Table 1. Demographic characteristics and distribution of risk factors in diabetic patients with and without CAD

Parameter	Diabetic patients with CAD (n=141)	Diabetic patients without CAD (N=369)	*P Value
Sex(M/F)	76/65(53.9%/46.0%)	170/199(46.0%/53.9%)	0.113
Age(years)	60.2±8.6 [a]	58.6±7.5	0.25
Smoking	30(21.3%)	71(19.2%)	0.266
Diabeticduration (years)	13.52±6.6	13.94±10.7	0.59
Systolic (mmHg)	144.8±22.1	136.4±20.7	<0.001
Diastolic (mmHg)	89.5±10.6	84.7±10.3	<0.001
F.B.S (mg/dL)	205.2±66.7	201.3±61.9	0.53
Cholesterol (mg/dL)	245.1±119.7	206.2±60.1	<0.001
Triglyceride (mg/dL)	193±83.5	170.5±67.5	0.004
H.D.L (mg/dL)	42.5±8.9	44.5±10.6	0.036
L.D.L (mg/dL)	126±34.1	118.35±10.5	0.015
V.L.D.L (mg/dL)	39.6±12.4	35.59±10.5	0.001
BMI	27±4.8	26.24±4.0	0.078

*a.Data are presented as mean±SD. Comparisons were made using student's t test (for continuous variables). Statistically significant; *P<0.05.*

Table 2. The distribution of ACE genotype in Diabetic patients with and without CAD

	CAD patients	Patients without CAD
ACE genotypes		
I/I	(n=21, 14.9%)	(n=95, 25.7%)
I/D	(n=69, 48.9%)	(n=180, 48.8%)
D/D	(n=51, 36.2%)	(n=94, 25.5%)
	$\chi2$=9.387, df=2, p=.009	
I/D+D/D	(n=120, 85.1%)	(n=174, 74.3%)
	$\chi2$=6.8, df=1, p=.009	
ACE alleles		
I	(n=111, 39.4%)	(n=370, 50.1%)
D	(n=171, 60.6%)	(n=368, 49.9%)
	$\chi2$=9.5, df=1, p=.002	

Note: The distribution and comparisons of genotype frequencies were made using $\chi2$ analysis.

Table 3. Odds ratios of ACE and VEGF genotypes and alleles with respect to I/I or I and C/C or C respectively, in Diabetic patients with and without CAD

	CAD patients	Patients without CAD
ACE genotypes		
I/I	Reference group (n=21)	Reference group (n=95)
I/D	1.73(1.32- 2.74, p=0.049,n=69)	n=180
D/D	2.45(1.37- 4.4, p=0.003, n=51)	n=94
(I/D+D/D)	1.98 (1.18- 3.3, p=0.01, n=51)	n=274
ACE alleles		
I	Reference group (n=111)	Reference group (n=370)
D	1.55 (1.2- 2.1, p=0.001,n=171)	n=368

49.9% in the control group. In addition, we found that the ACE D allele increases the risk of CAD in individuals 1.55-fold.

Consistent with data obtained from different studies[23-26], we found that ACE D increases the risk of CAD in CAD patients. Vaisi-Raygani, et al.[27], in-

dicated that, The angiotensin converting enzyme D allele is an independent risk factor for early onset coronary artery disease. Acartürk et al.[28], indicated that, the I/D polymorphism of ACE gene (carrying D allele) is an independent risk factor for CAD in the studied Turkish population.

In contrast, several investigators were not able to find an association between the ACE D allele and the CAD in the populations that they were investigating[18,29,30]. Shafiee, et al.[18], showed that, Angiotensin converting enzyme DD genotype not associated with increased risk of coronary artery disease in the Iranian population. Agerholm-Larsen et al.[31], which investigated the association in a case-referent study as well as in retrospective cohort study; no significant differences in incident myocardial infarction (MI) or any other manifestation of ischemic heart disease, between genotype classes were found.

The insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene is considered an important genetic determinant of CAD and hypertension[32, 33].

The mechanism by which the ACE I/D genotypes may predispose an individual to the development of CAD and MI remains unclear. ACE D allele has been correlated to increase ACE that is responsible for the conversion of angiotensin I to the peptide precursor angiotensin II that induces aldosterone production. Elevation of circulating levels of aldosterone influences on arterial hypertension, cardiac fibrosis and, consequently, both diastolic dysfunction and ventricular remodeling, is implicated in the pathogenesis of atherosclerosis[34].

These observations emphasize the importance of geographical location and ethnic background of the subjects in the study of ACE genotype and association with CAD. These findings stress the necessity of considering ethnic factors in the assessment of genetic risk identifiers. Thus, additional analysis is needed to clarify the real contribution of ACE to the development of CAD in different Iranian populations.

Conclusion

The insertion/deletion polymorphism of ACE gene (carrying D allele) is an independent risk factor for coronary artery disease in T2DM individuals.

Because of the small sample size in the present study and because Iranians show wide genetic diversities, additional analysis on a larger sampling is needed to clarify the contribution of ACE D allele to the development of CAD in different Iranian populations and various ethnic groups in the world.

References

1. *Kharrazi H, Vaisi-Raygani A, Sabokroh AR, et al. Association between apolipoprotein E polymorphism and coronary artery disease in the Kermanshah population in Iran. Clin Biochem 2006; 39: 613–6.*
2. *Vaisi-Raygani A, Rahimi Z, Nomani H, et al. The presence of apolipoprotein ε4andε2 alleles augments the risk of coronary artery disease in type 2 diabetic patients. Clin Biochem 2007; 40: 1150–6.*
3. *Vaisi Raygani A, Rahimi Z, Entezami H, et al. Butyrylcholinesterase K variants increase the risk of coronary artery disease in the population of western Iran. Scand J Clin Lab Invest 2008; 68: 123–9.*
4. *Vaisi Raygani A, Rahimi Z, Tavilani H, et al. Butyrylcholinesterase K variant and the APOE-ε4 allele work in synergy to increase the risk of coronary artery disease especially in diabetic patients. Molec Bio Rep 2010; 37: 2083–91.*
5. *Vaisi Raygani A, Tavilani H, Zahrai M, et al. Serum butyrylcholinesterase activity and phenotype associationswith lipid profile in stroke patients. Clin Biochem 2009; 42: 210–4.*
6. *Nikzamir A, Nakhjavani M, Golmohamadi T, et al. Association of angiotensinconverting enzyme geneinsertion/deletion polymorphismwithmetabolic syndrome in Iranians with type 2 diabetes mellitus. Arch Iranian Med 2008; 11: 3–9.*
7. *Hatmi ZN, Tahvildari S, Gafarzadeh Motlag A, et al. Prevalence of coronary artery disease risk factors in Iran: a population based survey. Cardiovasc Disord 2007; 7: 1–5.*
8. *Imazio M, Bobbio M, Bergerone S, et al. Clinical and epidemiological characteristics of juvenile myocardial infarction in Italy: the GISSI experience. G Ital Cardiol 1998; 28: 505–12.*
9. *Egred M, Viswanathan G, Davis GK. Myocardial infarction in young adults. Postgrad Med J 2005; 81: 741–5.*
10. *Movva S, Alluri RV, Komandur S, et al. Relationship of angiotensin-converting enzyme gene polymorphism with nephropathy associated with type 2 diabetes mellitus in Asian Indians. J Diabetes Complications 2007; 21: 237–241.*
11. *Rahimi Z, Felehgari V, Rahimi M, et al. The frequency of factor V Leiden mutation, ACE gene polymorphism, serum ACE activity and response to ACE inhibitor and angiotensin II receptor antagonist drugs in*

Iranians type II diabetic patients with microalbuminuria. Mol Biol Rep 2010; 38: 2117–2123.

12. *Cambian F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359-641.*
13. *Arfa I, Abid A, Nouira S et al. Lack of association between the angiotensin-converting enzyme gene (I/D) polymorphism and diabetic nephropathy in Tunisian type 2 diabetic patients. J Renin Angiotensin Aldosterone Syst 2008; 9: 32–36*
14. *Glenn KL, Du ZO, Eisenmann JC, et al. An alternative method for genotyping of the ACE I/D polymorphism. Mol Biol Rep 2009; 36: 1305–1310*
15. *Sayed-tabatabaei FA, Oostra BA, Isaacs A, et al. ACE polymorphisms, Circ. Res. 2006; 98: 1123– 1133.*
16. *Akbulut T, Bilsel T, Terzi S, et al. Relationship between ACE gene polymorphism and ischemic chronic heart failure in Turkish population, Eur. J. Med. Res. 2003; 8: 247–253.*
17. *Cambien F, Evans A. Angiotensin I converting enzyme gene polymorphism and coronary heart disease, Eur. Heart J. 1995; 16: 13–22.*
18. *Shafiee SM, Firoozrai M, Salimi S, et al. Angiotensin converting enzymeDDgenotype not associatedwith increased risk of coronary artery disease in the Iranian population. Pathophysiology 2010; 17: 163–7.*
19. *American Diabetes Association.(2005) Diagnosis and classification of diabetes mellitus. Diabet Care 28: S37–42.*
20. *Friedewald WT, Levy RI and Fredrickson DS .Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499–502.*
21. *Tiret L, Rigat B,Visvikis S, et al. Evidence, fromcombined segregation and linkage analysis, that a variant of the angiotensin 1 converting enzyme(ACE) gene controls plasma ACE level. Am J Hum Genet 1992; 51: 197–205.*
22. *Umbach D.M, Weinberg C.R. Designing and analyzing case–control studies to exploit independence of genotype and exposure, Stat. Med. 1997; 16: 1731–1743.*
23. *Arbustini E, Grasso M, Fasani R, et al. Angiotensin converting enzyme gene deletion allele is independently and strongly associated with coronary atherosclerosis and myocardial infarction. Br HeartJ 1995; 74: 584-591.*
24. *Dzimiri N, Basco C, Moorji A, et al. Angiotensin-converting enzyme polymorphism and the risk of coronary heart disease in the Saudi male population. Arch Pathol Lab Med 2000; 124(4): 531–4.*
25. *Hoshida S, Kato J, Nishino M, et al. Increased angiotensin-converting enzyme activity in coronary artery specimens from patients with acute coronary syndrome. Circulation 2001; 103: 630–3.*
26. *Zintzaras E, Raman G, Kitsios G, et al. Angiotensin-converting enzyme insertion/ deletion gene polymorphic variant as a marker of coronary artery disease: a meta-analysis. Arch Intern Med 2008; 168: 1077–89.*
27. *Vaisi-Raygani A, Ghaneialvar H, Rahimi Z, et al. The angiotensin converting enzyme D allele is an independent risk factor for early onset coronary artery disease. Clinic Biochem 2010; 43: 1189–1194.*
28. *Acartürk E, Attila G, Bozkurt A, et al. Insertion/ Deletion Polymorphism of the Angiotensin Converting Enzyme Gene in Coronary Artery Disease in Southern Turkey. Biochem and Mol Bio 2005; 38: 486-490.*
29. *Purnamasari D, Widjojo B, Antono D, et al. ACE Gene Polymorphism and Atherosclerotic Lesion of Carotid Artery Among Offsprings of Type 2 Diabetes Mellitus. Acta Medica Indonesiana 2012; 44: 128-134.*
30. *Mannami T, Katsuya T, Baba S, et al. Low potentiality of angiotensin-converting enzyme gene insertion/deletion polymorphism as a useful predictive marker for carotid atherogenesis in a large general population of a Japanese city: the Suita study. Stroke 2001; 32: 1250–6.*
31. *Agerholm-Larsen B, Nordestgaard B.G, Tybjaerg-Hansen A. ACE gene polymorphism in cardiovascular disease: meta-analyses of small and large studies in whites, Arterioscler. Thromb. Vasc. Biol. 2000; 20: 484–492.*
32. *Tiret L, Bonnardeaux A. Synergistic effects of angiotensin-converting enzyme and angiotensin II type 1 receptor DD genotype of the gene polymorphisms on risk of myocardial infarction. Lancet 1994; 344: 910–3.*
33. *Jeunemaitre X, Ledru F and Battaglia S. Genetic polymorphisms of the reninangiotensin system and angiographic extent and severity of coronary artery disease: the CORGENE study. Hum Genet 1997; 99: 66–73.*
34. *Yang Q, Khoury MJ. Evolving methods in genetic epidemiology III. Gene environment interaction in epidemiologic research. Epidemiol Rev 1997; 19: 33–43.*

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Challenges of family doctors and health professionals during the patient interviews

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Abstract

Aim: To identify the most frequent challenges during patient encounters.

Methods: One day prior to each workshop, we asked one major question, "For you, what are the first three challenges in a patient encounter?"

Results: Of the 721 participants, 367 (50.9%) were female, 354 (49.1%) were male, 401 (55.6%) were medical and dentistry students, 145 (20.1%) were physician assistants, 94 (13.0%) were family doctors, 81 (11.2%) were residents. The participants identified 78 challenges grouped in 14 categories: The most frequent challenges were communication problems (862, 42.4%), patient related conditions (405, 19.9%), breaking bad news (193, 9.5%), organizational problems (175, 8.6%), patients with high expectations (138, 6.8%), doctors' inadequate knowledge (94, 4.6%), language problems (45, 2.2%), family anger (33, 1.6%), and legal problems (24, 1.2%). Total 154 male (43.5%) and 152 (41.4%) female indicated communication as their first challenge. Communication was stated 39.0%, patient related problems were noted 18.8%, organizational problems 12.4% in the family doctors. Of the interns, communication was reported 32.5%, breaking bad news was reported 17.9%, doctors' inadequate knowledge 7.7%. Total 55.2% of the other medical students and 52.1%, of the dentistry students reported communication. In the physician assistants, communication was noted 59.1%, organizational problems 21.4%, patient related problems 11.4%. Responses of the residents: communication 35.1%, patient related problems 18.2%, 15.6% organizational problems.

Conclusion: Challenges centered on communication.

Key words: Patient encounter, communication, professional - patient relations, physician patient relations, challenges, health professionals, family medicine, general practitioner.

Background and aim

According to the physicians involved in a study, they perceived one sixth of patient encounters to be "difficult".[1] According to the study, patient characteristics that suggest the likelihood of difficult encounters include the presence of depressive or anxiety disorders, more somatic symptoms, and greater symptom severity. However, the patients were not responsible for all difficult encounters. The physician's attitudes about care, fatigue, stress, and burnout can create circumstances in which physicians are responsible for the difficult interaction.[2]

Many doctors think that their personality and character traits play a part in the fact that they perceive their difficult patients in the way that they do. Therefore it is appropriate to talk about the difficult encounter and not just the difficult patient. A difficult patient for one doctor may not necessarily be difficult for another. Doctors with poorer psychosocial skills perceive more of their medical encounters as difficult.[3, 4, 5, 6, 7, 8] Patients may become angry in response to suffering caused by illness, medical error, adverse life events, or simply as a result of being a patient.[9]

Research on the "difficult" patient generally defines the problem exclusively from the physician's perspective. "Difficult" patients are typically described as "those who raise negative feelings within the clinician," presumably due to behaviors deemed "inappropriate" in a treatment setting. The clinician's experience of frustration, anxiety, guilt, or dislike in interactions with the patient frames the research that explores the causal explanation of the dysfunctional dynamic. The conclusion reached by most studies is that a set of patient-centered problems or flaws accounts for the inappropriate behavior. The most commonly attributed cause of the "difficult" patient is the presence of a psychiatric disorder. The difficult or frustrating patient often has unrecognized psychiatric

problems. The candidates for such psychiatric diagnoses range from depression and anxiety to comorbid psychopathology, hostility, aberrant drug behavior, and chronic noncompliance.[10] Difficult patients can also occur because the patient may have challenging medical problems to diagnose or treat; or the patient may have changing symptoms; or the patient's complaints do not match known syndromes, which may indicate a somatization disorder.[11] It is useful to distinguish between difficulty due to challenging patient behavior and that due to a challenging disease or system problems.[7]

Our aim was to identify the most frequent challenges faced by health professionals during patient encounters through the design of a workshop. In our study, health professionals included family doctors, residents, physician assistants, and medical and dentistry students.

Methods

Since October 2009, the authors, who are members of the faculty of the Department of Family Medicine at the Medical School of Ondokuzmayis University, organized one-day workshops. The workshops focused on the challenges associated with the patient-physician/ health professional encounter. Workshop participants included: family doctors, medical and dentistry students, physician assistants, and residents of the University hospital located in Samsun and Trabzon, Turkey. To date, we have conducted 48 workshops with 15 participants in each.

One day prior to each workshop conducted between October 2009 and October 2012, we provided an open-ended questionnaire to each person signed up for the workshop. The questionnaire consisted of one major question, which was, "For you, what are the first three challenges in a patient encounter?" The content of each workshop was dynamic in that each workshop was organized according to the answers provided by that group of attendees. The workshops are composed of brain storming, discussions, learning from the colleague experiences, scenarios and presentations. The years of medical experience and gender of each workshop participant were noted.

The participants identified a total of 78 different challenges. After the workshops, we grouped the responses into 14 themes. The themes were based on groupings contained in the literature[5, 6, 7, 13, 14, 15, 16] and most common responses. Percentages of these challenges and the statistical evaluation by chi-square test according to the gender and occupation are presented in this study.

Results

Of the 721 participants, 367 (50.9 %) were female, 354 (49.1 %) were male, 401 (55.6 %) were students, 145 (20.1 %) were physician assistants, 94 (13.0%) were family doctors, and 81 (11.2%) were residents.

Of the 401 students, 251 were interns, 39 were second or third year medical students, and 104 were dentistry students. Of the group, a total of 626 (including students) work in the university, 94 in primary health care and one in the state hospital.

Of the 81 residents, their specialties were: internal medicine (27), gynecology (16), family medicine (13), infectious diseases (8), thoracic diseases (5), surgery (3), thoracic surgery (2), neurosurgery (2), cardiology (2), urology (1), orthopedics (1), and plastic surgery (1).

The participants identified a total of 78 different challenges (Table 1). The authors grouped these challenges in 14 main categories: communication problems, patient related conditions, organizational problems, family anger, patient with high expectations, breaking bad news, doctors' inadequate knowledge, homecare, legal problems, unknown medical history, greater healthcare use, irrational demands, very important patient (VIP) and language problems. Tables 2, 3 and 4 list the three major groups of challenges noted by the participants.

Of the 721 participants, we received 2033 responses that fell into the first three areas of challenge. The most frequent challenges noted by the participants were communication problems (862, 42.4%), patient related conditions (405, 19.9%), breaking bad news to the patient (193, 9.5%), organizational problems (175, 8.6%), patients with high expectations (138, 6.8 %), doctors' inadequate knowledge (94, 4.6%), language problems (45, 2.2%), family anger (33, 1.6 %), and legal problems (24, 1.2%). The other responses are listed in Tables 2, 3 and 4.

Table 1. Challenges in patient interviews identified by the health professionals and groups by the authors

1. Communication problems	Communication problems Aggressive patient Reluctant patient Prejudiced patient Impatient patient Misunderstanding Insensitive Yelling patient Patients without empathy Insisting patient Personal problems with the patient Irresponsible patient Unsatisfied patient Non-compliance Disrespectful patient Rambling patient Doctor being influenced Patient's lack of trust of the doctor Hypochondriac patient Quiet patient Phone consultation Patient with a gun
2. Patient related conditions	Feeling him/herself insufficient Uneducated patient Disabled patient Patients without relative Serious chronically ill patient Serious oncologic patient Serious psychiatric patient New born and young infant with serious problem Unconscious patient Mental retardation Drug abuser Dirty patient Serious trauma Neurologic patient Complicated patient Communicable disease Dementia Sexual problems Serious chest pain Serious emergencies Pregnant patient Gender discriminating patient
3. Breaking bad news	Breaking bad news
4. Organizational problems	Documentation problems Patients who don't want to wait Patients who do not obey the rules Limited time Technical problems Working environment Appointment problems Financial problems of the patient Patient without health insurance Requirement of seeing all patients Inadequate conditions for physical examination
5. Patient with high expectations	Patient with high expectations Private patient Patients using the Internet to diagnosis their condition Patient who thinks that he/she knows everything
6. Language problems	Language problems
7. Doctors' inadequate knowledge	When doctor's knowledge is insufficient Patient whose treatment is useless Triage Not knowing referral criteria Serious complications
8.Patient relative	Family anger
9. Legal problems	Legal problems Forensic case Patient rejecting treatment Patient talking about patient rights
10. VIP patient	Relative of a healthcare provider Doctor as a patient
11. Irrational demands	Ask a prescription for another person Irrational prescription, sickness certification demand
12. Homecare patient	Homecare patient
13. Unknown medical history	Unknown medical history
14. Greater healthcare use	Greater healthcare use

Table 2. Primary challenges noted by the participants

Challenges	Frequency	Percentage
Communication problems	306	42.4
Patient related problems	153	21.2
Breaking bad news	113	15.7
Organizational problems	60	8.3
High expectations	33	4.6
Language problems	15	2.1
Family anger	14	1.9
Doctors' inadequate knowledge	11	1.5
Irrational demands	6	0.8
Legal problems	4	0.6
Unknown medical history	2	0.3
Homecare	2	0.3
VIP patient	2	0.3
Total	721	100.0

Table 3. Secondary challenges noted by the participants

Challenges	Frequency	Percentage
Communication problems	289	40.1
Patient related problems	137	19.0
Breaking bad news	44	6.1
Organizational problems	59	8.2
High expectations	67	9.3
Language problems	14	1.9
Family anger	24	3.3
Doctors' inadequate knowledge	39	5.4
Irrational demands	3	0.4
Legal problems	7	1.0
Unknown medical history	5	0.7
Homecare	1	0.1
VIP patient	3	0.4
No response	29	4.0
Total	721	100.0

Of the 354 male participants, 154 (43.5%) indicated communication as their first challenge, and the 152 (41.4%) female participants indicated the same. There was no statistical significant difference between two gender regarding communication problems (p=0.9). While 85 (23.2%) women indicated patient related problems as first, 68 (19.2%) men indicated the same. There was no statistical significant difference between two gender regarding patient related problems (p=0.16). A total of 45 (12.3%) women indicated organizational problems as their primary challenge, and this number was 15 (4.2%) for the male participants. There was a statistical significant difference between two gender regarding the response of organizational problems (p=0.000).

Table 4. The third level of challenges noted by the participants

Challenges	Frequency	Percentage
Communication problems	267	37.0
Patient related problems	115	16.0
Organizational problems	56	7.8
Doctors' inadequate knowledge	44	6.1
High expectations	38	5.3
Breaking bad news	36	5.0
Language problems	16	2.2
Family anger	15	2.1
Legal problems	13	1.8
Greater healthcare use	8	1.1
VIP patient	8	1.1
Irrational demands	3	0.4
Homecare	1	0.1
No response	101	14.0
Total	721	100.0

Of the 758 interns, communication problems were reported 246 (32.5%) times, breaking bad news was reported 136 times (17.9%), doctors' inadequate knowledge was reported 58 times (7.7%), and patient with high expectations was reported 58 times (7.7%). Responses also noted language problems (35, 4.6%) and organizational problems (27, 3.6%). The other responses (198, 26.1%) indicated patients with high expectations, legal problems, unknown medical history, greater healthcare use, irrational demands and VIP patient.

From the other 39 second and third year students, we received 105 responses. There were 58 (55.2%) stating communication problems, 24 (22.9 %) indicating patient related problems, and 10 (9.5%) noting breaking bad news as a challenge. The other 13 (12.4%) responses noted organizational problems, doctors' inadequate knowledge, legal problems, patients with high expectations and language problems.

From the 104 dentistry students, we received 307 responses. In this group, 160 (52.1%) were communication problems, 96 (31.3%) were pa-

tient related problems, and 16 (5.2%) were breaking bad news. Doctors' inadequate knowledge was reported 16 (5.2%) times, patient with high expectations was reported 8 (2.6%) times, organizational problems were noted 8 (2.6%) times, and greater healthcare use was stated 3 (1.0%) times.

From the 145 physician assistants, we received 350 responses. Communication problems were noted 207 (59.1%) times, organizational problems 75 (21.4%) times, and patient related problems were identified 40 (11.4%) times. The remaining 28 (8.0%) responses noted family anger, patient with high expectations, VIP patient, and language problems.

Of the 94 family doctors participating in the workshops, we received 282 responses. Communications problems were stated 110 (39.0%) times, patient related problems were noted 53 (18.8%) times, organizational problems were stated 35 (12.4%) times, and patients with high expectations was noted 33 (11.7%) times. The remaining 51 (18.1%) responses were irrational demands, homecare, unknown medical history, language problems, legal problems, greater healthcare use and VIP patient.

From the 81 residents, we received 231 responses. Their responses were as grouped as follows: communication problems 81 (35.1%), patient related problems 42 (18.2%), 36 (15.6%) organizational problems, 30 (13.0%) breaking bad news, family anger 20 (8.7%), patients with high expectations.11 (4.8%). The remaining responses were doctors' inadequate knowledge, irrational demands, legal problems, language problems, and VIP patients.

When the participants are evaluated as students, physician assistants, residents and family doctors regarding communication problems, the responses on communication problems were seen more frequent in the student (32.5%, 55.2%, 52.1%) and physician assistant (59.1%) groups than the family doctors (39.0%) and residents (35.1%) (p= 0.00).

Breaking bad news responses were more frequent in the students and residents than the others (p=0.00). Dentistry students and second or third year medical students identified patient related conditions more frequent than the other groups (p= 0.00).

Physician assistants, family doctors and residents identified organizational problems more frequent than the students (p= 0.004).

Discussion

Our study is practice-based as we have organized workshops based on ascertaining challenges encountered during patient interactions. The content of each workshop is dynamic in that it is based on the needs of the participants. There are many curriculums to teach the challenges.[9, 17]An essential skill to learn is the ability to coach the patient to be your ally in his/ her care. Certain behaviors will change when a trusting relationship is established.[17] In our study, patient's lack of trust of the doctor was a problem that we grouped this under communication problems.

In a study with medical students, mean comparisons for stress reactions to uncertainty, indicated no significant differences based on gender or specialty interest.[19] In another study, specialty groups with a higher incidence of reported disruptive behaviors included anesthesia, cardiology, hospitalists, orthopedics, trauma, and obstetrics/ gynecology. Female physicians were less likely to be reported[20] as having disruptive behaviors. In a study with primary care resident physicians, female physicians provided increased patient centered care to their patients. The greatest amount of patient centered care was seen in the female patient-female physician gender dyad.[21] We could not find any significant difference between the two genders regarding communication problems.

We found that communication problems decreased with the number of years of experience. Our results indicated that males and females responded that their biggest challenge was providing answers to patients who had unreasonable expectations in the similar rates. Despite significant clinical and research efforts aimed at recognizing and managing "difficult" patients, such patients remain a frustrating experience for many clinicians. This is especially true for primary care residents, who are required to see a significant volume of patients with diverse and complex problems, but who may not have adequate training and life experience to enable them to deal with problematic doctor-patient situations.[17]

In a study of 102 respected family physicians with ages ranging from 35 to 85, two main groups of difficult patients were reported based on behavior and medical problems. As their most common response, these physicians noted multiple

complaints from these difficult patients.[22] In the same study, one participant claimed that he had never had a difficult patient.[22] In our study, all of the participants had experienced challenges. Physicians with less experience may be more at risk of perceiving the encounter as difficult. Younger physicians reported more challenging patient encounters than older colleagues.[23,24] Our study also showed that communication problems were noted more frequently by less experienced healthcare providers, especially students.

Various organizational factors can also contribute to difficult patient encounters.[25] The realities of today's health care system require that practitioners see more patients in order to stay in business. This means that there is less time for the physician to interact with each patient and less time in which to develop a relationship.[26] Increasing administrative requirements for health care delivery encroach on time spent with patients. The curriculum for trainees recommends time management.[27] Environmental factors such as chaotic or noisy waiting rooms, hurried staff, and even a lack of patient privacy can establish a detrimental atmosphere.[17]In our study, administrative and environmental problems were common in family doctor, resident and physician assistant groups.

Additional stress may evolve from language barriers and cultural issues that inhibit clear doctor-patient communication.[17] In another study, some of the challenges in improving physician-patient concordance, and in turn the trust between doctor and patient, have been brought out in the qualitative inputs from focus group discussions among selected physicians. The doctors felt handicapped by language and cultural barriers in communicating with the patient.[28] Language problems were important factors in our study especially for the interns.

Sometimes the problem may be in dealing with someone other than the patient, such as a caregiver, spouse, or parent who insists on being in the examining room.[17] In our study, we found family anger to be a common problem.

In our study, we found that younger physicians or interns had communication problems more frequently, and in particular, breaking bad news was their significant problem. Breaking bad news is a difficult task, but one that physicians have to do. Education of the doctors in techniques for delivering bad news seems to be beneficial.[12, 29] Death and dying, breaking bad news, painful patient, interactions with uncooperative patients, or patients who behaved inappropriately were the most common problems indicated in a study conducted among second year medical students.[30] Poor communication of end-of-life matters by interns is all too common and has been shown to be associated with poor patient-care outcomes, including inadequate pain and symptom relief.[31]

Number of hours worked per week by the physician has been found to be associated with increased frustration with patients.[32] The younger physicians in our study were residents and interns who had prolonged work hours. Stressed physicians may also view more patients as being difficult.[32] Older physicians were less likely to report high frustration with patients, perhaps because of greater clinical experience or a more flexible and humanistic approach to patient care. It is also possible that there are fewer highly frustrated physicians in the older age group because these physicians are less likely to continue practicing clinical medicine.[32] In our study residents, family doctors and interns identified more organizational problems probably because of prolonged work hours.

In our study, one major communication problem was the patient's lack of trust of the doctor. This lack of trust has been reported in another study especially in situations where the patients had drug use issues.[33]

In a study with family doctors, the most commonly reported sources of conflict were requests for sickness certification, benzodiazepines (typically for insomnia or anxiety), and antibiotics for respiratory tract infections. The idea of 'daily battles' featured more prominently in accounts from trainees compared to more experienced GPs. Similar to those, our results showed that family doctors were faced with irrational demands from patients such as providing a sickness certification when they were not ill and demanding inappropriate prescription drugs. Conflict between the patient and the doctor resulted when the doctor refused to assist the patient under these circumstances.[34]

Good communication is central to clinical practice and has positive effects in the daily interaction between dentist and patient: In this context, a growing interest could be observed in dentistry to inte-

grate the teaching of communication in the curricula and to define learning targets for the different levels of qualification in pre- and post-graduate clinical education. In our study, communication problems were commonly noted by dentistry students.[35]

As the educational process for family physicians and physician assistants has evolved, several trends have emerged. The patient safety movement has led to an increased focus on inter-professional teamwork, which underscores the importance of improved communication among different providers.[36] There has been increasing emphasis on the importance of inter-professional collaborative practice as it improves the quality of patient care and safety, enhances the accessibility and continuity of care, and helps to decrease conflict, redundancy and staff turnover.[37] Factors associated with the health care provider's role that are attributable to disparities are threefold: 1) bias or prejudice, 2) clinical uncertainty when caring for minority patients and 3) assumptions made by the clinician about minority health care needs. These three factors all display a failure of "intercultural competence," defined as "the ability to think and act in inter-culturally appropriate ways. In the same study this skill was found to be improved after a curriculum in physician assistant students.[38] In our workshops, challenges in communication was the most important challenge for the physician assistants and language problems also were identified and we focused on communication in this group.

It was found that the residents' perceptions of their patients' anxiety and depression predicted difficulty ratings independently of the patients' objectively measured medical or psychiatric status.[39] Our results show that the residents and students perceived patients with serious psychiatric problems as challenging that we grouped this problem as patient related problems.

More than three-fourths of providers felt that older patients with dementia were difficult to manage, whereas fewer than half shared that view with respect to their heart disease patients.[40] In our study, health care providers perceived elderly patients and patients with dementia as challenging.

A significant proportion of primary care physicians report diagnostic difficulty involving at least 5% of their patients. The doctors indicated patient factors (education, communication) or organizational problems including workload.[41] Our results show that the family doctors and residents perceive organizational problems as challenging conditions.

Some limitations of our study deserve consideration. The basis of our data is the information provided by various healthcare providers responding to our questionnaire. We are unable to determine how much of the variation in reported challenging interviews is due to differences between patient loads and how much is due to differences between healthcare providers themselves.

Conclusion

In a study of characteristics of patient encounters that challenge medical students' provision of patient-centered care, students identified the importance of gathering a more complete social history after the workshop.[42] Understanding the features that contribute to these difficult patient encounters provides important needs assessment data for curricula design to address clinical challenges. The most common challenges centered on communication skills in our study. To further investigate this issue based on the results of this study, we organized a new course which we will recommend to the family doctors and health professionals at Ondokuzmayis University hospital in Samsun.

Previous presentation

The study was presented in KAHEKON 2011 National Family Medicine Conference in October 2011 in Trabzon- Turkey an took the best poster award of the conference.

The study was also presented in WONCA Europe 2012 Family Medicine Conference, in July 2012, Vien- Austria.

References

1. *Jackson JL, Kroenke K. [Difficult patient encounters in the ambulatory clinic: Clinical predictors and outcomes]. Arch Intern Med 1999; 159: 1069-75.*

2. *Hull SK, Broquet K. [How to manage difficult patient encounters]. Fam Pract Manag 2007; 14(6): 30-4.*

3. *Garrison D, Lyness JM, Frank JB, Epstein RM. [Qualitative analysis of medical student impressions of a narrative exercise in the third-year psychiatry clerkship]. Acad Med 2011; 86(1): 85-9.*

4. *Mathers NJ, Jones N, Hannay D. [Heartsink patients: A study of their general practitioners]. Br J Gen Pract 1995; 45: 293-6.*

5. *Brown JB, Stewart M, Weston WW, ed. In: Challenges and Solutions in Patient- Centered Care. Radcliffe Medical Press, Oxon: UK; 2002.*

6. *Magill MK. Difficult doctor- patient relationships. In: Saultz JW, Textbook of Family Medicine. McGraw-Hill press; New York; 2000.p.699-712.*

7. *Loder E. [The approach to the difficult patient]. Handb Clin Neurol 2010; 97: 233-8.*

8. *Bickley LS, Szilagy PG. Adapting your interview to specific situations. In: Bates' Guide To Physical examination and history taking, 10th ed. Lippincott Williams and Wilkins press, Philadelphia; 2009.p.75-80.*

9. *Braun IM, Barsky AJ. Approach to the angry patient. In: Primary Care Medicine. Office Evaluation and Management of the Adult Patient. 6th ed. Goroll AH, Mulley AG, ed. Lippincott Williams Wilkins and Wolters Kluwer press, Philadelphia; 2009.p.1481-3.*

10. *Fiester A. [The "difficult" patient reconceived: An expanded moral mandate for clinical ethics]. Am J Bioeth. 2012; 12(5): 2-7.*

11. *Lynch DJ, McGrady AV, Nagel RW, Wahl EF. [The patient-physician relationship and medical utilization]. Prim Care Companion J Clin Psychiatry 2007; 9(4): 266-70.*

12. *Dikici MF, Yaris F, Cubukcu M. [Teaching medical students how to break bad news: A Turkish experience]. J Cancer Educ. 2009; 24(4): 246-8.*

13. *Bower DJ, Young S, Larson G, Simpson D, Tipnis S, Begaz T, Webb T. [Characteristics of patient encounters that challenge medical students' provision of patient-centered care]. Acad Med 2009; 84(10): 74-8.*

14. *Patient encounters of the difficult kind. The Quarterly Journal for Health Care Practice and Risk Management 2009; 10: 1-4.*

15. *Travaline JM, Ruchinskas R, D'Alonzo GE Jr. [Patient-physician communication: Why and how]. J Am Osteopath Assoc 2005; 105(1): 13-8.*

16. *Ritter RH, Reis MD, Rascoe TG. Interviewing techniques. In: Textbook of Family Medicine. 8th ed. Rakel RE, Rakel DP, ed. Elsevier Saunders, Philadelphia; 2011.p.166-75.*

17. *Shapiro J, Lie D. [Using literature to help physician-learners understand and manage "difficult" patients]. Acad Med 2000; 75(7): 765-8.*

18. *Romano SE, Schwenk TL. The challenging patient encounter. In: Essentials of Family Medicine 5th ed. Sloane PD, Slatt LM, Ebell MH, Jacques LB, Smith MA ed. Lippincott Williams and Walters Kluwer, press Philadelphia; 2008; p.31- 8.*

19. *Evans L, Trotter DR, Jones BG, Ragain RM, Cook RL, Prabhu FR, Linton KS. [Epistemology and uncertainty: A follow-up study with third-year medical students]. Fam Med 2012; 44(1): 14-21.*

20. *Goettler CE, Butler TS, Shackleford P, Rotondo MF. [Physician behavior: Not ready for 'Never'land]. Am Surg 2011; 77(12): 1600-5.*

21. *Bertakis KD, Azari R. [Patient-centered care: The influence of patient and resident physician gender and gender concordance in primary care]. J Womens Health (Larchmt). 2012; 21(3): 326-33.*

22. *Elder N, Ricer R, Tobias B. [How respected family physicians manage difficult patient encounters]. J Am Board Fam Med 2006; 19(6): 533-41.*

23. *Breuner CC, Moreno MA. [Approaches to the difficult patient/parent encounter]. Pediatrics 2011; 127(1): 163-9.*

24. *Steinmetz D, Tabenkin H. [The 'difficult patient' as perceived by family physicians]. Fam Pract 2001; 18(5): 495-500.*

25. *Goold SD, Lipkin M. [The doctor-patient relationship: Challenges, opportunities and strategies]. J Gen Intern Med 1999; (1 suppl); 26-33.*

26. *Haas LJ, Leiser JP, Magill MK, Sanyer ON. [Management of the difficult patient]. American Family Physician 2005; 72(10): 2063-8.*

27. *Dugdale DC, Epstein R, Pantilat SZ. [Time and the patient-physician relationship]. J Gen Intern Med 1999; 14(suppl 1): 34-40.*

28. *Banerjee A, Sanyal D. [Dynamics of doctor-patient relationship: A cross-sectional study on concordance, trust, and patient enablement]. J Fam Community Med 2012; 19: 12-9.*

29. *Dikici MF, Yaris F, Artiran Igde F. Communication with cancer patients in family medicine. In: Surbone A, Zwitter M, Rajer M, Stiefer R. Springer ed. New Challenges in Communication with Cancer Patients, Springer, New York; 2013; p.263-74.*

30. *Dyrbye LN, Harris I, Rohren CH. [Early clinical experiences from students' perspectives: A qualitative study of narratives]. Acad Med 2007; 82(10): 979-88.*

31. *Supe AN. [Interns' perspectives about communicating bad news to patients: A qualitative study]. Educ Health (Abingdon) 2011; 24(3): 541.*

32. *E. E. Krebs, J. M. Garrett and T. R. Konrad. [The Difficult doctor? Characteristics of physicians who report frustration with patients: An analysis of survey data]. BMC Health Services Research. 2006; 128(6): 1-8.*

33. *Bezreh T, Laws MB, Taubin T, Rifkin DE, Wilson IB. [Challenges to physician-patient communication about medication use: A window into the skeptical patient's world]. Patient Prefer Adherence 2012; 6: 11-8.*

34. *Walter A, Chew-Graham C, Harrison S. [Negotiating refusal in primary care consultations: A qualitative study]. Fam Pract 2011; 29(4): 488-96.*

35. *Haak R, Rosenbohm J, Koerfer A, Obliers R, Wicht MJ. [The effect of undergraduate education in communication skills: A randomised controlled clinical trial]. Eur J Dent Educ 2008; 12(4): 213-8.*

36. *Kruse J. [Overcoming barriers to interprofessional education: The example of the joint position statement of the physician assistant education association and the society of teachers of family medicine]. Fam Med 2012; 44(8): 586-8.*

37. *Solomon P, Salfi J. [Evaluation of an interprofessional education communication skills initiative]. Educ Health (Abingdon) 2011; 24(2): 1-10.*

38. *Huckabee MJ, Matkin GS. [Examining intercultural sensitivity and competency of physician assistant students]. J Allied Health 2012; 41(3): 55-61.*

39. *De Marco MA, Cítero VA, Nogueira-Martins MC, Yazigi L, Wissow LS, Nogueira-Martins LA, Andreoli SB. [Medical residency: Factors relating to "difficulty in helping" in the resident physician-patient relationship]. Sao Paulo Med J 2011; 129(1): 5-10.*

40. *Harris DP, Chodosh J, Vassar SD, Vickrey BG, Shapiro MF. [Primary care providers' views of challenges and rewards of dementia care relative to other conditions]. J Am Geriatr Soc 2009; 57(12): 2209-16.*

41. *Sarkar U, Bonacum D, Strull W, Spitzmueller C, Jin N, López A, Giardina TD, Meyer AN, Singh H. [Challenges of making a diagnosis in the outpatient setting: A multi-site survey of primary care physicians]. BMJ Qual Saf 2012; 21(8): 641-8.*

42. *Bower DJ, Young S, Larson G, Simpson D, Tipnis S, Begaz T, Webb T. [Characteristics of patient encounters that challenge medical students' provision of patient-centered care]. Acad Med 2009; 84(10 Suppl): 74-8.*

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Effects of traditional Chinese medicine Epimedium on the micro-inflammatory state of non-uremic patients with chronic renal failure

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Abstract

Objective: To explore the effects of traditional Chinese medicine Epimedium on the micro-inflammatory state of non-uremic patients with chronic renal failure (CRF).

Methods: 120 non-uremia CRF patients who received treatment in the outpatient or were hospitalized in our hospital from January 2011 to June 2012 were selected, which were divided into two groups according to different treatment methods: treatment group and control group (n=60). The patients in the control group were routinely given antihypertensive drugs, erythropoietin and symptomatic treatment; those in the treatment group were additionally taken 10g raw Epimedium after infusion in hot water on the basis of the conventional treatment for the control group, once a day. The treatment lasted for two weeks. 60 healthy subjects receiving physical examination in the examination center of our hospital in the same period were selected as the normal control. The levels of serum creatinine (Scr), amyloid protein A (SAA) and high-sensitivity C-reactive protein (hs-CRP) were measured in both the treatment and control groups before and after treatment respectively, and meanwhile the endogenous creatinine clearance rate (Ccr) was calculated, which were compared with the results of the normal control group.

Results: There were no statistically significant differences in the levels of serum Scr and Ccr in the two groups between before and after the treatment ($P>0.05$ for both); the serum SAA and hs-CRP levels in the two groups were significantly increased compared with the normal control group before the treatment ($P<0.05$ for both); the differences in the levels of serum SAA and hs-CRP in the control group were not statistically significant between before and after the treatment ($P>0.05$ for both), while the levels of serum SAA and hs-CRP in the control group after the treatment were significantly decreased compared with before the treatment and the control group ($P<0.05$ for all). The correlation analysis showed that the levels of serum SAA and hs-CRP in the 60 cases of non-uremia CRF patients before treatment were positively correlated ($r = 0.866$, $P<0.05$).

Conclusion: The micro-inflammatory state exists in non-uremic patients with CRF. Epimedium can enhance the micro-inflammatory state of the patients, but can not improve the kidney function significantly.

Key words: CRF, non-uremic patient, Epimedium, micro-inflammatory state.

Introduction

Chronic renal failure (CRF), also known as chronic renal insufficiency, refers to the chronic progressive renal damage caused by various reasons, which is clinically manifested with the progressive development of chronic kidney disease (CKD) with metabolites retention, water and electrolyte imbalance and acid-base disturbance, eventually resulting in a group of clinical syndromes of end-stage renal disease [1]. Cardiovascular disease is the most common complication of CRF, and also serves as one of the leading causes of death. The occurrence and development of cardiovascular events is considered to have a close relationship with the inflammatory response [2]. Epimedium is a traditional Chinese medicine commonly used in the clinical treatment of CRF [3]. This plant belongs to berberidaceae brevicomum Maxim., as shown in Figure 1, with high medicinal values.

Figure 1. Structure of Epimedium

According to the traditional Chinese medicine, Epimedium is in acrid-sweet flavor and warm-natured, with channel tropism to liver and kidney, the attending functions of which include reinforcing the kidney yang, strengthening muscles and bones and expelling wind-damp. Epimedium is generally used for the treatment of infantile paralysis, neurasthenia and chronic bronchitis [4]. Its role in endocrine is mainly the androgen effect, which can improve the function of the immune system and cardiovascular system, affect the blood system, anti-osteoporosis, and promote the synthesis of DNA to affect the metabolism and other pharmacological effects [5]. The aforementioned pharmacological effects of Epimedium are involved in the occurrence and development process of micro-inflammation [6]. The purpose of this study is to explore the effects of Epimedium on the micro-inflammatory state in patients with non-uremic CRF.

Materials and Methods

Inclusion standards

1) The glomerular filtration rate (GRF) of all the patients were less than 90mL/min, which continued for more than three months; 2) all the patients were non-uremic patients not given hemodialysis treatment [135μmol/L<serum creatinine (Scr)<707μmol/L]; 3) the patients were in a stabilized condition, who had self-care ability, without obvious edema.

Exclusion standards

1) Patients combined with autoimmune diseases and cancer; 2) patients suffering from acute infection, active liver diseases, surgery and trauma recently; 3) patients taking glucocorticoids, immunosuppressive agents, lipid-lowering drugs and anticoagulants, e.g. aspirin.

Objects

120 non-uremic patients with CRF who received treatment in the outpatient or were hospitalized in our hospital from January 2011 to June 2012 were selected, all of whom met the diagnostic criteria revised on the Anhui Taiping Conference in June 1992 [7] and the above requirements for selection and exclusion. The patients were divided into two groups according to different treatment methods, 60 for each group. 60 patients of the treatment group included 23 males and 37 females, of whom the oldest was 76 years old, the youngest 20 years old, with the duration of 0.45 to 7.58 years. In this group, the upper arm muscle circumference was 16.43 to 24.01cm, body mass 42.98 to 70.11kg, body mass index 18.24 27.32kg/m^2, and there were 30 cases of chronic glomerulonephritis, 16 cases of hypertensive renal damage, 8 cases of polycystic kidney disease, 6 cases of chronic pyelonephritis. The patients in the control group included 25 males and 35 females, of whom ages were from 21 to 75 years old, with the duration of 0.39 to 7.67 years. In this group, the upper arm muscle circumference was 16.57 to 24.34cm, body mass 43.15 to 69.87kg, body mass index 17.66 to 27.12kg/m^2, and there were 32 cases of chronic glomerulonephritis, 15 cases of hypertensive renal damage, 7 cases of polycystic kidney and 6 cases of chronic pyelonephritis. 60 healthy subjects receiving physical examination in the examination center of our hospital in the same period were selected as the normal control, including 29 males and 31 females, aged from 20 to 75 years old. The differences in gender, age, upper arm muscle circumference, levels of body weight, body mass index, serum albumin and prealbumin were not statistically significant among the three groups (P>0.05 for all). There was statistical significance in the differences in the level of serum Scr and endogenous creatinine clearance rate (Ccr) among the three groups (P<0.05 for all), and the course of disease between the treatment group and the control group showed no significant difference (P>0.05) (Table 1).

Table 1. General information ($\bar{x}\pm s$)

Item		Normal control (n=60)	Control (n=60)	Treatment (n=60)
Gender	male	29	25	23
	female	31	35	37
Age (years old)		46.54±17.67	48.78±16.15	47.65±15.97
Course (year)		—	4.11±2.12	3.98±2.35
Body weight (kg)		54.67±7.81	55.74±7.92	55.92±7.16
BMI (Kg/m^2)		22.13±2.35	22.25±2.50	22.16±2.47
Upper arm muscle circumference (cm)		20.60±2.19	20.73±1.91	20.57±2.01
Serum Scr (**μmol**/L)		67.88±17.13	403.56±152.18	400.26±159.49
Ccr (mL/min)		96.53±9.05	31.07±16.12	34.07±22.10
Serum albumin (g/L)		42.27±3.15	37.38±3.02	36.99±2.82
Serum prealbumin (mg/L)		315.78±24.51	286.55±21.25	286.12±20.63

**P>0.05, △P<0.05, compared to the normal control group; #P>0.05, compared to the control group*

Table 2. Examination indicators before and after treatment ($\bar{x}\pm s$)

Item	Normal control (n=60)	Control (n=60)		Treatment (n=60)	
		Before	After	Before	After
Scr(**μmol**/L)	67.88±17.13	403.56±152.18	405.87±153.25	400.26±159.49	389.16±169.10
Ccr(mL/min)	96.53±9.05	31.07±16.12	31.05±15.18	34.07±22.10	34.36±21.54
SAA(**μg**/L)	575.16±219.87	3815.64±1503.77	3756.89±1478.32	3749.07±1545.70	2398.76±1182.03
hs-CRP(mg/L)	2.24±1.20	7.25±2.31	7.19±2.47	7.15±2.67	5.26±2.18

*#P>0.05, compared to the same group before treatment; **P<0.05, compared to the normal control group; *P<0.05, △P<0.05, △△P>0.05, compared to the same group*

Methods

The patients in the control group received conventional drug therapy with antihypertensive drugs (calcium channel blockers), erythropoietin and symptomatic treatment (excluding other drugs of detoxification and creatinine and urea nitrogen reduction, statins and antioxidants such as vitamin E , C, etc.); the patients in the treatment group were additionally given 10g Epimedium (produced by Henan Ancheng Bio-tech Co., Ltd.) after infusion in hot water on the basis of the conventional treatment for the control group, once a day. The treatment lasted for two weeks. Specimen collection: 3mL fasting cubital venous blood was drawn from the patients in the three groups in the morning and centrifuged at 1000r/min for 5min to get serum, which was preserved in -20 ℃ refrigerator for measurement. Detection methods: The automatic biochemical analyzer produced by China Perlong Medical Equipment Co., Ltd. was used to detect the serum Scr level by kinetic rate method; the level of serum amyloid protein A (SAA) was measured by ELISA with the Perlong Medical 9602A microplate reader; the automatic specific protein analyzer of Shenzhen Histrong HC988 Series was adopted to determine the level of serum high-sensitivity C-reactive protein(hs-CRP) by the rate nephelometry. The kits for this test were purchased from Wuhan Elaborate Biotechnology Co., Ltd. The above operations were carried out in strict accordance with the kit instructions. The value of Ccr was calculated according to the Cockcroft-Grault formula in literatures [8].

Statistical analysis

All data were analyzed by SPSS18.0. Measurement data were expressed as $\bar{x}\pm s$, and the two groups were compared by the t test. Numeration data were compared by the χ^2 test. The normal distribution data were subjected to Pearson correlation analysis, and the abnormal distribution data were subjected to Spearma's rank correlation analysis. $P<0.05$ was considered statistically significant.

Results

There were no statistically significant differences in the levels of serum Scr and Ccr in the two groups between before and after the treatment (P>0.05 for both); the serum SAA and hs-CRP levels in the two groups were significantly increased compared with the normal control group before the treatment (P<0.05 for both); the differences in the levels of serum SAA and hs-CRP in the control group were not statistically significant between before and after the treatment (P>0.05 for both), while the levels of serum SAA and hs-CRP in the control group after the treatment were significantly decreased compared with before the treatment and the control group (P<0.05 for all) (Table 2).

The correlation analysis showed that the levels of serum SAA and hs-CRP in the 120 cases of non-uremia CRF patients before the treatment were positively correlated (r = 0.866, P <0.05) (Figure 2).

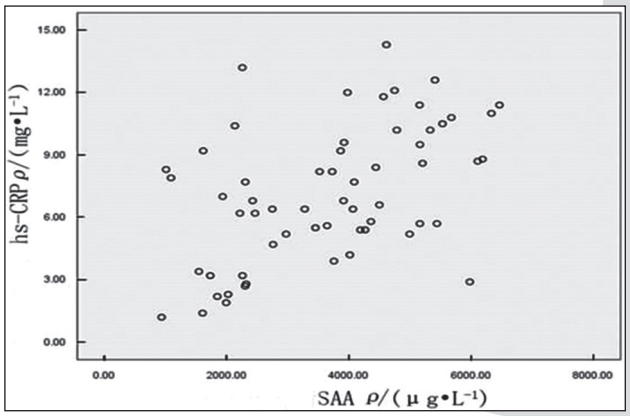

Figure 2. Correlation between serum SAA and hs-CRP before treatment

Seven patients in the treatment group suffered from diarrhea, nausea and vomiting, which were significantly improved after the additive amount of Epimedium was reduced. No serious adverse reactions were found in the control group.

Discussion

The view that the "micro- inflammatory state" existed in uremic patients was put forward by Schömig. M et al. for the first time in 2000[9], thus opening up the way of "micro-inflammatory" research on chronic nephrosis. Later, some studies proved that the micro-inflammatory state existed in the majority of CRF patients [10], mainly manifested with the increase of positive acute reactive proteins, such as C reactive protein (CRP), SAA and ferritin, etc. and the increase of inflammatory cytokines, such as IL-1, IL-6 and TNF-α, etc. and the decrease of negative acute reactive proteins, such as serum albumin, prealbumin, transferrin and retinol binding protein, etc. [11]. Micro-inflammatory state is not only possible to expedite progressive renal failure of CKD patients [12], but also involved in the occurrence of cardiovascular diseases, anemia and other complications [13], which greatly improves the mortality. Therefore, it is of great help for the prognosis of patients to detect sensitive micro-inflammatory indexes with a high specificity and intervene in the micro-inflammatory state in an early stage [14]. CRP is an early- recognized marker which is important in reflecting micro-inflammatory state in vivo. Lezaic [15] studied the relationship between CRP and the occurrence of cardiovascular complications and cardiovascular and cerebrovascular events (including cardiac failure, unstable angina pectoris, acute myocardial infarction, sudden cardiac death and cerebrovascular events) in non-dialysis patients with CRF, and considered that, with the increase of CRP level, the fatality rate and incidence of cardiovascular and cerebrovascular events of non-dialysis CRF patients increased significantly. Devins et al. [16] made follow-up visits to predialysis patients for one year, monitoring serum CRP and other indicators, in which there were about 35% of patients underwent elevated CRP level and the patients with the history of cardiovascular diseases showed more significant increase in CRP level. The studies have suggested that inflammatory state exists generally in CRF patients [17], and that high level of serum CRP indicates an ongoing inflammatory state which is related to hypoalbuminemia, low erythrogenin reaction and high rate of hospitalization occurring in patients after dialysis treatment. However, although CRP may reflect the inflammatory state in the body, it lacks sensitivity to the micro-inflammatory state in CRF patients. With the improvement of experimental means, trace amounts of CRP existing in blood may be detected currently, i.e. hs-CRP, which can monitor the micro-inflammatory state

of patients more accurately [18] and evaluate the degree of micro-inflammation. The results of this study showed that the hs-CRP level of patients in the control and treatment groups before treatment was significantly increased compared with the normal control group, also confirming that micro-inflammatory state exists in non-uremic patients with CRF, which is consistent with literature report [19]. SAA is an acute phase reaction protein researches have been greatly concerned in recent years, mainly synthesized by hepatocytes. There are trace amounts of SAA in normal serum. When the organism is in the inflammatory state, however, SAA can be rapidly increased to 1000~2000 times of the normal value [20]. Similar to CRP, SAA can also be expressed and produced under the stimulation of cytokines, such as IL-1α, IL-6, TNF-α and IFN-α, and is more sensitive than CRP[21]. The research results of Powell RL et al. showed that SAA level significantly increases in the early stage of CRF, SAA mainly metabolizes through enzymatic degradation as it is not affected by gender, age and other factors, with the half-life period of only 50min; therefore, it can reflect the imperceptible changes of inflammatory response level in the body in a rapid and sensitive way. They proposed that SAA is expected to be a favorable index for the early diagnosis of CRF micro-inflammatory reactive state [22]. This study also observed the changes of SAA in each group and showed that the level of SAA in healthy subjects was significantly lower than that of non-uremic patients with CRF, and the serum SAA and hs-CRP levels in non-uremia CRF patients before treatment were positively correlated ($r=0.866$, $P<0.05$), which further provides evidence for the presence of micro-inflammatory state in patients with non-uremic CRF.

Epimedium is one of the traditional Chinese medicines. Since Epimedium was used for the treatment of patients with CRF [23], researches on its renal protective effect have emerged in endlessly. An increasing number of laboratory and clinical studies have confirmed that Epimedium has such functions as immune regulation, anti-inflammation, inhibition of platelet aggregation, improvement of microcirculation, free radical scavenging, inhibition of proliferation of glomerular mesangial cells and fibroblasts and confrontation against renal interstitial fibrosis, thus significantly slowing down the process of renal insufficiency and glomerulosclerosis [24]. This study found that, after treatment given to non-uremia CRF patients with Epimedium, there were significant changes in the decrease of serum hs-CRP and SAA levels compared with the control group only given conventional treatment, and the differences in the serum hs-CRP and SAA levels were statistically significant between the treatment and control groups after treatment ($P<0.05$ for both), suggesting that Epimedium can also improve the micro-inflammatory state of non-uremia CRF patients. In addition, this study observed the changes of serum Scr and Ccr levels in both groups of non-uremic patients with CRF before and after treatment with Epimedium. The two indicators of the treatment group showed a tendency of improvement, but the differences between the two groups had no statistical significance before and after treatment ($P>0.05$ for both), indicating renal function not significantly improved by Epimedium [25]. It was inferred that this might be related to the large range of variation of Scr and Ccr levels in non-uremia CRF patients selected by the Institute and the relatively short time of treatment with Epimedium. In short, the micro-inflammatory state exists in non-uremic patients with CRF, for whom the levels of hs-CRP, SAA and other indicators should be detected in an early stage so as to learn about the micro-inflammatory state in a timely manner and give intervention treatment as soon as possible. Moreover, although this study considered that Epimedium can improve the micro-inflammatory state of CRF patients, due to the small size of samples, relatively short study time, lack of multi-center studies and failure to observe adverse reactions of long-term use of Epimedium, it is necessary to extend the observation time in the future and further study the influence of Epimedium on the micro-inflammatory state and its protective effect on renal function of non-uremia CRF patients, so as to provide more evidences for slowing down the progress in non-uremic patients with CRF.

References

1. *Korish AA, Arafah MM. Catechin combined with vitamins C and E ameliorates insulin resistance (IR) and atherosclerotic changes in aged rats with chronic renal failure (CRF). Arch Gerontol Geriatr 2008; 46: 25-39.*

2. *Girndt M, Seibert E. Premature cardiovascular disease in chronic renal failure (CRF): A model for an advanced ageing process. Exp Gerontol 2010; 45: 797-800.*

3. *Pan Y, Kong LD, Li YC, Xia X, Kung HF, Jiang FX. Icariin from Epimedium brevicornum attenuates chronic mild stress-induced behavioral and neuroendocrinological alterations in male Wistar rats. Pharmacol Biochem Behav 2007; 87: 130-140.*

4. *Shen ZY, Huang JH, Wu B. [Activating effect and mechanism of epimedium on endogenous stem cells]. Zhongguo Zhong Xi Yi Jie He Za Zhi 2009; 29: 251-254.*

5. *Huang W, Sun W, Wang Y. Isolation and molecular characterisation of flavonoid 3'-hydroxylase and flavonoid 3', 5'-hydroxylase genes from a traditional Chinese medicinal plant, Epimedium sagittatum. Gene 2012; 497: 125-130.*

6. *Rostoker G, Griuncelli M, Loridon C, Bourlet T, Illouz E, Benmaadi A. Modulation of oxidative stress and microinflammatory status by colloids in refractory dialytic hypotension. BMC Nephrol 2011; 12: 58.*

7. *Wang HY, Zheng FL, Liu YC,et al. [Primary glomerular disease classification and treatment of its diagnostic criteria symposium Minutes] Zhonghua Nei Ke Za Zhi 1993; 32: 131-134.*

8. *Lau W, Pye D. Changes in corneal biomechanics and applanation tonometry with induced corneal swelling. Invest Ophthalmol Vis Sci 2011; 52: 3207-3214.*

9. *Schömig M, Eisenhardt A, Ritz E. The microinflammatory state of uremia [J]. Blood Purif, 2000; 18(4): 327-332.*

10. *Cao W, Liu JH, Zhang H, Zhang L, Zhang LY, Pan MM. [Effect of acupoint injection on erythropoietin resistance in patients with chronic renal failure]. Zhongguo Zhen Jiu 2010; 30: 891-895.*

11. *Rogowski O, Shapira I, Bassat OK, Chundadze T, Finn T, Berliner S, et al. Waist circumference as the predominant contributor to the micro-inflammatory response in the metabolic syndrome: a cross sectional study. J Inflamm (Lond) 2010; 7: 35.*

12. *Merino A, Portoles J, Selgas R, Ojeda R, Buendia P, Ocana J, et al. Effect of different dialysis modalities on microinflammatory status and endothelial damage. Clin J Am Soc Nephrol 2010; 5: 227-234.*

13. *Garibotto G, Sofia A, Procopio V, Villaggio B, Tarroni A, Di Martino M, et al. Peripheral tissue release of interleukin-6 in patients with chronic kidney diseases: effects of end-stage renal disease and microinflammatory state. Kidney Int 2006; 70: 384-390.*

14. *Pu C, Yang YB, Sun QL. [Effects of Salvia miltiorrhiza on oxidative stress and microinflammatory state in patients undergoing continuous hemodialysis]. Zhongguo Zhong Xi Yi Jie He Za Zhi 2006; 26: 791-794.*

15. *Lezaic V, Tirmenstajn-Jankovic B, Bukvic D, Vujisic B, Perovic M, Novakovic N, et al. Efficacy of hyperphosphatemia control in the progression of chronic renal failure and the prevalence of cardiovascular calcification. Clin Nephrol 2009; 71: 21-29.*

16. *Devins GM, Mendelssohn DC, Barre PE, Taub K, Binik YM. Predialysis psychoeducational intervention extends survival in CKD: a 20-year follow-up. Am J Kidney Dis 2005; 46: 1088-1098.*

17. *Zhang R, Li S. COX-2 as a novel target of CRF family peptides' participating in inflammation. Biochem Biophys Res Commun 2009; 382: 483-485.*

18. *Tsimogiannis KE, Tellis CC, Tselepis AD, Pappas-Gogos G, Bakola MS, Tsimoyiannis EC, et al. alpha-Defensins and hsCRP levels in inflammatory response of standard and laparoendoscopic single-site cholecystectomy. Surg Endosc 2012; 26: 627-631.*

19. *Musial K, Zwolinska D, Polak-Jonkisz D, Makulska I, Miler M. [Antibodies against heat shock proteins (HSP) in children with chronic renal failure (CRF)--preliminary results]. Przegl Lek 2006; 63 Suppl 3: 60-62.*

20. *Wu TL, Tsai IC, Chang PY, Tsao KC, Sun CF, Wu LL, et al. Establishment of an in-house ELISA and the reference range for serum amyloid A (SAA): complementarity between SAA and C-reactive protein as markers of inflammation. Clin Chim Acta 2007; 376: 72-76.*

21. *Yoshizaki K. Pathogenic role of IL-6 combined with TNF-alpha or IL-1 in the induction of acute phase proteins SAA and CRP in chronic inflammatory diseases. Adv Exp Med Biol 2011; 691: 141-150.*

22. *Powell RL, Zhao J, Konings FA, Tang S, Ewane L, Burda S, et al. Circulating recombinant form (CRF) 37_cpx: an old strain in Cameroon composed of diverse, genetically distant lineages of subtypes A and G. AIDS Res Hum Retroviruses 2007; 23: 923-933.*

23. *Cheng QL, Chen XM, Shi SZ. [Effects of Epimedium sagittatum on immunopathology and extracellular matrices in rats with chronic renal insufficiency]. Zhonghua Nei Ke Za Zhi 1994; 33: 83-86.*

24. *Liao HJ, Chen XM, Li WG. [Effect of Epimedium sagittatum on quality of life and cellular immunity in patients of hemodialysis maintenance]. Zhongguo Zhong Xi Yi Jie He Za Zhi 1995; 15: 202-204.*

25. *Chen X, Zhou M, Wang J. [Effect of epimedium sagittatum on soluble IL-2 receptor and IL-6 levels in patients undergoing hemodialysis]. Zhonghua Nei Ke Za Zhi 1995; 34: 102-104.*

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Treatment of brucellosis and liver toxicity

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Abstract

Introduction: Brucellosis is an important public health problem world-wide, especially in developing countries. Besides hepatic involvement of the disease, hepatitis may ocur due to antimicrobial drugs. The aim of this study is to investigate the hepatotoxicity of the treatment regimens of brucellosis.

Patients and methods: In this study, 126 cases diagnosed with brucellosis who admitted to the Department of the Infectious Diseases and Clinical Microbiology, from November 2010 to August 2011, were evaluated, treated and followed up for hepatoxicity, prospectively.

Results: The coombs STA test was positive in 126 cases (100 %). Focal involvement was present in 27(21.4%) cases. The most frequent involvement was osteoarticular involvement observed in 22 cases (17.5%). Genitourinary system involvement was present in 3 patients (2.4%). Hepatic involvement was present in 2 patients (1.6%).

Conclusion: Brucellosis may cause serious morbidity in humans and it continues to be a major health problem in Turkey.

Key words: Brucellosis, treatment, hepatotoxicity.

Introduction

Brucellosis is a multisystemic infection and a zoonosis. Brucellae, is a facultative intracellular, non-motile, non-encapsulated, gram-negative coccobacillus. Brucellosis can be seen in all regions of the world[1,2]. It can affect virtually any tissue or organ, and various literature reports describe Brucella infection of the respiratory system, the skin, and the gastrointestinal tract. Brucellosis can be seen at any age but most commonly in old males[2]. It is an important public health problem world-wide, especially in developing countries[3]. Transmission of Brucellae from infected animals to humans occurs by consumption of infected dairy products, direct contact with the infected tissue (urine, body secretions and pregnancy materials) or blood, and inhalation of infectious aerosal particles[3,4]. Some rare cases were reported which had infection by breastfeeding and sexual contact. Fever, night sweats, weight loss and myalgia are common symptoms. Many various symptoms can be seen according to the involved organ or system in brucellosis[4].

The incubation period is between one and four weeks. Brucellosis is a multi- systemic disease which was the most frequent cause of granulomatosis reactions in reticuloendothelial system[5-7].

Liver involvement results in mild to moderate elevation in liver enzymes. Hepatic involvement has been reported in around 2-3% of brucellosis cases[8-10]. The World Health Organization(WHO) recommends doxycyline 200 mg/day and rifampin 600-900 mg/day combination therapy for six weeks[11]. Drug tolerability is very important in the therapeutic options of human brucellosis. The adverse drug effects in antibrucellar regimens are generally mild and well tolerated[12]. The aim of this study was to evaluate hepatotoxicity of treatment regimens of brucellosis.

Materials and methods

A total of 126 cases with the diagnosis of brucellosis admitted to our clinic, the Department of Infectious Disease and Clinical Microbiology of Batman State Hospital from November 2010 to August 2011. This study received local ethics committee approval. This study was carried out as a prospective study. The patients were evaluated with their clinical findings, medical history and laboratory findings, as well as complications and clinical outcomes.

The informations of patients included demographic data, serologic, treatment, laboratory findings, hepatit markers and abdominal USG findings. Brucellosis was diagnosed on the basis of following criteria:

The symptoms, such as fever, chills, night sweats, hepatomegaly, splenomegaly, headache, backache, generalized pain, arthralgia, and malasie. Those symptomps were supported by the detection of spesific antibodies at significant titers and/or the demonstrations of an at least four-fold rise in antibody titer in sera taken 2 or 3 weeks later. Significant titers were those determined to be ≥ 1/160 in standard tube agglutination test (STA)[13].

B.abortus S99 antigens (Pendik Veterinary Control and Research Institution, Istanbul, Turkey) were used for STA. Serologic tests were all carried out according to previously described techniques. Screening was done by slide agglutination or Rose Bengal plate agglutination test (Pendik Veterinary Control and Research Institution, Istanbul, Turkey). Serologic evaluation was performed using Brucella agglutination tests [Rose Bengal Slide agglutination test and the Wright standard tube aglutination (STA) test][14]. During the study period we could not perform blood culture for all patients because of lack of equipments in Batman State Hospital. All cases underwent routine laboratory tests and also tested for HbsAg and anti-HCV.

Abdominal Ultrasound was performed in all cases for hepatic complications. The other radiologic examinations, such as plain X-ray, scrotal USG and magnetic resonance imaging (MRI) were performed when needed for further investigation of the complications.

Complication or focal form is defined as presentation of symptoms or physical signs of infection at a particular anatomic site in a patient with brucellosis. Osteoarticular involvement was considered when there were inflammatory signs (erythema, heat, edema, swelling, pain, or functional disability) in any peripheral joint, or when there is unrelieved pain at rest together with radiological alterations, evaluated independently by both the radiologist and the clinician. Hepatic involvement is defined as a five-fold increase (≥ 200 IU/I) in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels without any other etiologic explation, and/or total bilirubin levels of over 2,5 g/dl. Hematologic involvement is defined as hematologic abnormalities in laboratory findings.

Treatment and follow-up

Patients were treated with following antibiotic combinations according to their age, clinical findings and complications. The regimens including: oral doxycycline (200 mg/day)+oral rifampin (600 mg/day) + intramuscular streptomycin (1 g/day) or oral doxycycline (200 mg/day) +oral rifampin (600 mg/day). When the treatment regimens were changed, the regimens included oral ciprofloxacin (1000 mg/day) + oral doxycycline (200 mg/day). These antimicrobials were given minimum 6 weeks. When required, the duration of therapy was extended and data were recorded. All patients were followed up for at least 1-2 weeks during hospitalizations. Outpatients were called for control visits at 2-week intervals. The mean follow up period was six weeks.

At control visits, complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), liver enzymes (ALT, AST, ALP, GGT, T.B., D.B.) were examined. These tests were repeated at each further control visit until full recovery. Hepatotoxicity was defined as AST or ALT >3x upper limit of normal (ULN) with symptoms or AST/ALT >5x ULN without symptoms. Further, " moderate hepatotoxicity" was defined as AST/ALT 5 to 10x ULN. "Severe hepatotoxicity" was defined as AST/ALT >10x ULN [15]. Additional patient data were also evaluated, including history of liver disease. When the brucellosis treatment was undertaken with baseline elevation of AST or ALT, patients were closely monitored.

Results

This study was conducted with 126 people brucellosis. Of the total 126 patients, 62(49.6%) were female and 64(50.4%) were male. The mean patient age was 34.64 ±15.22 years. The most common laboratory findings were high CRP levels (40.2%), high ESR levels (43.0%), and anemia (42.1%). Lymphocytosis was determined in 17 patients (13.5%), anemia in 53 (42.1%), thrombocytopenia in 12(9.5%), leukopenia in 5(4.0%), leukocytosis in 4(3.2 %) patients.

The STA test was positive in all cases, with titers ranging from 1/160 to 1/ 1280. Six cases (4.7%) who had 1/160, 11 cases (8.7 %) who had 1/320 and 109 (86.5 %) who had ≥ 1/640 titers. The most common laboratory findings are shown in Table 1.

Table 1. Laboratory findings

Laboratory findings		Patients/ study population (n:126)
Lymphocytosis (>50%) %		13.5
Anemia	Hematocrit<%36 for females	42.1
	Hematocrit <%42 for males	40.3
Thrombocytopenia (<150x10^3/mm^3)		9.5
Leukopenia (<4000/mm^3)		4,0
Leukocytosis (>11 000/mm^3)		3,2
Elevated ESR (> 20 mm/h)		43.0
High CRP (>0,8 mg/dL)		40,2
STA 1/160		4,8
STA 1/320		8,7
STA ≥ 1/640		86,5

Abbrevations:ESR; erytrocyte sedimentation rate, CRP; C-reactive protein, STA; standart tube agglutination.

Focal involvement was detected in 27 (21.4%) cases. The most frequent involvement was osteoarticular involvement with 22 cases (17.5%). Osteoarticular involvement included peripheral arthritis 1 (0.8%), sacroiliitis 15 (11.9%) and spondylitis 6 (4.8%) cases. Monoarthritis was seen in only 1 patient. Sacroiliitis was unilateral in 15 cases but bilateral in none of patients. Splenomegaly in 19 (15.1%) patients and hepatomegaly in 30 (23.8%) patients were detected with abdominal USG screening.

Genitourinary system involvement was present in 3 (2.4%) patients. Unilateral epididymoorchitis was detected in all cases. Clinical manifestations-organ involvement of patients are shown in table 2.

Table 2. Focal involvement of brucellosis

Organ involvement	Patients % (n:216)
Osteoarticular -Peripheral arthritis -Spondylitis -Sacroiliitis	17.46 - 0.79 - 4.76 - 11.91
Genitourinary- Epididymoorchitis	2.38
Gastrointestinal- Hepatitis	1.59

HBsAg was positive in 5(4.0%) patients, whereas noone had positive anti-HCV. Hepatic involvement was present in 2 patients (1.6%). These patients had three-fold ALT and AST levels. These patients' viral hepatitis markers were negative [Hbs Ag: (-) and anti-HCV: (-)] with normal abdomen USG. Ten (7.9%) patients had high AST levels in before treatment. Four of them had two-fold AST levels, but 6 had one-fold AST levels. They had only high AST levels, but they had normal ALT levels. Initial regimens were administered to total of 126 patients. The treatment regimens were doxycycline (200 mg/day PO), rifampin (600 mg/day PO) and streptomycin (1000 mg/day IM for the first 3 weeks) in 25 patients, and doxycycline (200 mg/day P.O.), rifampin (600 mg/day PO) were given to 101 patients.

The treatment regimens were changed in 14 (11.1%) patients, findings of these patients are shown in table 3. Rifampin was stopped, doxycycline (200 mg/day PO), ciprofloxacin (1000 mg/day) was given 14 patients. Three (2.4%) patients had elevations in AST or ALT of >5 times ULN. Three patients had AST/ALT values above the ULN at baseline and moderate hepatotoxicity. These patients had negative hepatit markers. Two patients had splenomegaly in abdomen USG and 1 patient had normal abdomen USG. Five patients with HBV infection had normal level liver function test. Four patients had normal abdomen USG but 1 patient had hepato-splenomegaly in abdomen USG. Rifampin - doxycycline was given to these patients. In second week of treatment patients had normal liver function tests including ALT, AST. Serum ALT-AST levels did not elevate in any of them, so treatment regimens of them were not changed.

Discussion

Brucellosis is a multisystemic disease in which any organ or body system can be involved[5-7]. Although brucellosis has been controlled or eradicated in many developed countries, it still remains a major health problem in developing countries particularly in the Mediterranean and Middle East[3,8]. Human brucellosis has a wide clinical spectrum and presents various diagnostic difficulties[5]. The symptoms are nonspecific; nevertheless, the majority of patients was complaining about fever, anorexia, headache, malaise, sweats, backache and arthralgia[4].

Brucellosis is the most common zoonotic infectious disease in the World, affecting more than

Table 3. Patients findings who need change in antibiotic regimen

Patients		Findings before treatment						At 2nd week of treatment	
Age	Sex	AST	ALT	Abdomen US	Drug use	HBsAg	Antihcv	AST	ALT
44	F	17	12	N	-	-	-	41	49
57	F	22	27	N	-	-	-	56	61
19	F	29	23	N	-	-	-	57	76
22	M	73	77	N	-	-	-	74	75
58	M	47	23	HSM	-	-	-	84	56
23	F	39	41	HSM	-	-	-	85	145
31	M	23	50	HM	-	-	-	88	169
23	F	82	55	N	-	-	-	103	115
30	M	26	37	N	-	-	-	108	113
15	F	56	28	N	-	-	-	120	60
64	M	23	20	N	-	-	-	136	92
22	M	67	54	SM	-	-	-	292	236
49	M	108	48	SM	-	-	-	416	271
30	M	88	95	N	-	-	-	610	548

Abbrevations: F; female, M; male, US; ultrasound, HSM; hepatosplenomegaly, SM; splenomegaly, HM; hepatomegaly; N; normal.

500.000 people each year[16]. Appropriate antimicrobial therapy and duration of the treatment will reduce morbidity, prevent complications and decrease relapses[17]. The first systematic study of the epidemiology of brucellosis was performed by Çetin et al. between 1984 and 1987 in Turkish patients[18].

Brucellosis is more prevalent in the 15-35 years age group in endemic countries[19]. In this study, 62 (49. 6%) of the patients were female and 64 (50.4 %) were male. The patients' ages ranged between 15 and 65 years and mean age was 34±15.22. Brucellosis can ocur in any age group but the majority of cases are found in young men between the ages of 20 and 40 years. Generally this is related to the professional danger of young men[20]. Anemia, leukopenia, thrombocytopenia, increased CRP and elevated liver enzymes were the most prevalant laboratory abnormalities seen in brucellosis. Increased ESR and lymphomonocytosis were also observed in brucellosis[19-21]. In our study, anemia was determined in 53 (42.1%) patients, lymphocytosis in 17 (13.5%), thrombocytopenia in 12 (9.5%), leukopenia in 5 (4.0%), leukocytosis 4 (3.2%), elevated ESR in 52 (43.0%) and CRP level in 49 (40.2%) patients.

When bacterial isolation is not possible, serology is the preffered method for the diagnosis of brucellosis[19-21]. STA test positivity was reported as 94,1% by Buzgan et al.[22] and as 98.8% by Demiroglu et al.[23]. The STA test and coombs STA test were positive in 100% of our study population. When the STA test is found negative, serum was tested with coombs antiseum.

Brucella infection may effect any tissue or organ in the body. Organ involvement can be presented as a complication or as focal involvement. The locomotor, genitourinary, gastrointestinal, hematologic, cardiovascular, respiratory and central nervous system are frequently affected in brucellosis[19,20]. Focal involvement have been reported in between 27.7-43.2%[8,24-26]. Focal involvement was observed in 27 (21.4%) patients in our study.

Osteoarticular involvement occurs in 20-85% of patients[17,18]. Osteoarticular involvement have been reported in between 9,3% and 79.5%[9,23,24,27-30]. In our study, osteartícular involvement was observed in 22 patients (17.5%). Osteoarticular involvement included peripheral arthritis in 1, sacroiliitis in 15 and spondylitis in 6 patients. The most frequent involvement was osteoarticular involvement in our study.

The liver is affected in majority of patients with brucellosis, because of being the largest organ of the reticuloendothelial system. Liver involvement presents as mild to moderate elevation of liver enzymes[20,21]. Hepatic involvement has been reported in between 2% and 3%[8,9,27]. In a study, liver enzyme elevation was observed in 24.8 % of the patients whereas clinical hepatitis was detected in only 2.7% of patients[22]. In our study, elevation of li-

ver enzymes was occured in 19 (15.1%) of patients and clinical hepatitis was observed in 2 of them.

In 2-10% of patients, urogenital involvement occurs, with unilateral epididymo-orchitis as the most common presentation[20-22]. In our study, genitourinary system involvement was occured in 3(2.4%) patients, presenting as epididymo-orchitis.

The standard regimens include doxycycline plus streptomycin or rifampin for brucellosis. In addition, a combination of one of these drugs with ciprofoxacin has also been used in recent years[31,32]. WHO recommends doxycycline 200 mg/day and rifampin 600-900 mg/day combination therapy for six weeks[11]. Doxycycline- rifampin was the preferred choice due to easy orl administration and cheaper cost. Doxycycline- streptomycin regimen was preferred as the therapy of choice for the treatment of osteoarticular complication of brucellosis[33,34]. Doxycycline- rifampin therapy protocol had been administered to 101(80.2%) patients in our study. Twenty five (19.8%) patients complicated with epididymoorchitis or osteoarticular involvement were treated by doxycycline-rifampin-streptomycin combination therapy.

Drug tolerability is very important in therapeutic options of human brucellosis. The adverse drug effects of antibrucellar regimens are mild and well tolerated. When doxycycline- rifampin was used, adverse reaction rate was reported as 12.9% by Hashemi et. al. Most of the adverse effects were classified as mild; these reactions were not severe enough to stop or modify the regimens[12]. In our study, rifampin was stopped in cases with liver toxicity, and ciprofloxacin-doxycycline was given patients. The treatment regimen was changed in 14 (11.1%) patients because of side effects.

In conclusion, brucellosis is still an important health problem. It is a multisystemic infectious disease with complications and various clinical presentations. Additionally, serological tests and bacterial isolation, non-spesific tests such as CRP and ESR can also be used in diagnosis and follow-up. WHO recommends 200 mg/day doxycyline and 600-900 mg/day rifampin combination therapy for six weeks. The adverse drug effects in antibrucellar regimens are mild and well-tolerated. Early reports of rifampin hepatotoxicity occured nearly 4 decades ago[35-37]. In our study, hepatotoxic adverse effects were classified as rarely. Hepatotoxicity associated with rifampicin may range from hyperbilirubinemia without hepatocellular demage to moderate elevations in transaminases or, rarely, clinically significant hepatitis[38-40].

References

1. *Görgülü A, Albayrak BS, Görgülü E, Tural O, Karaaslan T, Oyar O, et al. Spinal epidural abscess due to Brucella. Surg Neurol 2006; 66: 141-7.*
2. *Solera J, Lozano E, Martinez-Alfaro E, Espinosa A, Castillejos ML, Abad L. Brucellar spondylitis: review of 35 cases and literature survey. Clin Infect Dis 1999; 29: 1440-9*
3. *Young EJ. An overview of human brucellosis. Clin Infect Dis 1995; 21: 283-90*
4. *Young EJ. Brucella species. In: Mandell GL, Douglas JE, Bennett R, editors. Mandell, Douglas, and Bennett's principles and practice of infectious disease. 4 th ed. Oxford: Churchill Livingstone; 2000.p. 2386-91.*
5. *Monir Madkour M, Sharif HS, Abed MY, Al Fayez MA. Osteoarticular brucellosis: results of bone scintigraphy in 140 patients. Am J Radiol 1988; 150: 1101-5.*
6. *Glaskow MS. Brucellosis of the spine. Br J Surg 1976; 63: 283-8.*
7. *Bahar RH, Suhaili AR, Mousa AM, Nawaz MK, Kaddah N, Abdel-Dayem HM. Brucellosis: appearance on skeletal imaging. Clin Nucl Med 1988; 1; 102-6.*
8. *Colmenero JD, Reguera JM, Martos F, Sanchez De Mora D, Delgado M, Causse M, et al. Complications associated with Brucella melitensis infection: a study of 530 cases. Medicine (Baltimore) 1996: 75: 195-211.*
9. *Gür A, Geyik MF, Dikici B, Nas K, Cevik R, Sarac J, et al. Complications of brucellosis in different age groups: a study of 283 cases in Southeastern Anatolia of Turkey. Yonsei Med J 2003; 44: 33-44.*
10. *Ertek M, Yazgı H, Kadanalı A, Özden K, Taşyaran MA. Complications of Brucella infection among adults: an 18-year retrospective evaluation. Turk J Med Sci 2006; 36: 377-81.*
11. *WHO, Joint Food and Agriculture Organization, World Health Organization, FAO-WHO, Expert Committee on Brucellosis (sixth report). World Health Organ Tech Rep Ser 1986; 740: 56-57.*
12. *Hashemi SH, Gachkar L, Keramat F, Mamani M, Hajilooi M, Janbakhsh A, et al. Comparison of doxycyline-streptomycin, doxycyline-rifampin, and ofloxacin-rifampin in the treatment of brucellosis: a randomized clinical trial. International Journal of Infectious Diseases 16 (2012) e247-e251.*
13. *Young EJ. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. Rev Infect Dis 1991; 13: 359-72.*

14. *Alton GG, Jones LM, Pietz DE. Laboratory techniques in brucellosis, 2nd ed.,Geneva: World Health Organization; 1975.*

15. *ATS/CDC/IDSA Joint Statement. Treatment of tuberculosis. Am J Respir Crit Care Med 2003; 167: 603-62.*

16. *Papas G, Papadimitrious P, Akritidis N, Christou L,Tsianos EV. The new global map of human brucellosis. Lancet Infect. Dis.2006; 6: 91-9*

17. *Solera J, Martineza-Alfaro E, Espinaso A. Recognition and optimal treatment of brucellosis. Drugs 1997; 53: 245-56.*

18. *Çetin ET, Çoral B, Bilgiç A, Bilgehan E, Sipahioglu U, Gurel M. The incidence of brucellosis in human in Turkey. Doga-Turk J Med. Sci 1990; 14: 324-34.*

19. *Young EJ. Brucella species. In: Mandell GL, Bennet JE, Dolin R, editors. Principles and practice of infectious diseases. 6th ed., Philadelphia: Churchill Livingstone; 2005. 2669-72.*

20. *Doğanay M, Meşe Alp E. In: Topcu AW, Söyletir G, Doğanay M, editors. İnfeksiyon hastalıkları ve mikrobiyolojisi. 3rd ed.,Istanbul: Nobel Tıp Kitabevleri; 2008. 897-909.*

21. *Gotuzzo E, Celillo E, Brucella. In: Gorbach SI, Bartlett JG, Blacklow NR, editors. Infectious diseases. Philedalphia: Harcourt Brace Jovanovich Inc; 1992; 1513-8.*

22. *Buzgan T, Karahocagil MK, Irmak H, Baran AI, Karsen H, Evirgen O, et al. Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. International Journal of Inf. Dis. 14 (2010) e469-e478*

23. *Demiroğlu YZ, Turunç T, Alışkan H, Çolakoğlu S, Arslan H. Brucellosis: retrospective evaluation of the clinical, laboratory and epidemiological features of 151 cases. Mikrobiyol. Bul. 2007; 41: 517-527.*

24. *Aygen B, Doğanay M, Sümerkan B, Yildiz O, Kayabas Ü. Clinical manifestations, complications and treatment of brucellosis: a retrospective evaluation of 480 patients. Med Mal Infect 2002; 32: 485-93.*

25. *Örmen B, Türker N, Kaptan F, Ural S, Vardar İ, El S, et al. Retrospective evaluation of 104 cases with brucellosis. İzmir Atatürk Eğitim Hast Tıp Derg 2004; 42: 173-6.*

26. *Yüce A, Alp-Çavuş S, Yapar N, Çakır N. Brucellosis: Evaluation of 55 cases. Klimik Derg 2006; 19: 13-7.*

27. *Hacıbektaşoğlu A, Barut A, İnal A, Özer FT. Serologic and clinical characteristics of brucellosis in young adults and middle aged. In: Tümbay E, Hilmi S, Ang Ö, editors. Brucella and Brucellosis in man and animals. İzmir, Turkey: Ege University Press; 1991. 168.*

28. *Coşkun NA, Ural S, Müftüoğlu L, Kaptan F, Üremek H. An evaluation of 103 cases of brucellosis hospitalized in the 1985-91 period. Turk J Infect Dis 1993; 7: 283-7.*

29. *Turgut H, Hoşoğlu S, Aydın K, Arıtürk S. Clinical and laboratory findings in 98 patients. Med J Ege Univ 1991; 1: 153-7.*

30. *Barroso Garcia P, Rodriguez-Contreras Pelayo R, Gil Extremera B, Maldonado Martin A, Guilarro Heratas G, Martin Salguero A, et al. Study of 1595 brucellosis cases in the Almeria province (1972-1998) based on epidemiological data from disease reporting. Rev Clin Esp 2002; 202: 577-82.*

31. *Pappas G, Akritidis N, Bosilkovski M,Tsianos E. Brucellosis. N Engl J Med 2005; 352: 2325-36*

32. *Ersoy Y, Sönmez E, Tevfik MR, But AD. Comparison of three different combination therapies in the treatment of human brucellosis. Trop Doct 2005; 35: 210-2.*

33. *Ariza J, Bosilkovski M, Cascio A, Colmenero JD, Corbel MJ, Falagas ME, et al.; International Society of Chemotherapy; Institute of Continuing Medical Education of Ioannina. Prespectives for the treatment of brucellosis in the 21 st century: the Ioannina recommendations. PLoS Med 2007; 4: 1872-8.*

34. *Solera J, Martinez-Alfaro E, Espinosa A, Castillejos ML, Geijo P, Rodriguez-Zapata M. Multivariate model for predicting relapses in human brucellosis. J Infect 1998; 36: 85-92.*

35. *Lees AW, Asgher B, Hashem MA, Sinha BN. Jaundice after rifampicin. Brit J Dis Chest 1970; 64: 90-5.*

36. *Gabriel R. Rifampicin jaundice. BMJ 1971; 3: 1982.*

37. *Capelle P, Dhumeaux D, Mora M, Feldmann G, Berthelot P. Effect of rifampicin on liver function in man. Gut 1972; 13: 366-71.*

38. *Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, Nolan CM, et al.; ATS (American Thoracic Society) Hepatotoxicity of Antituberculosis Therapy Subcommittee. An official ATS statement: hepatotoxicity of antituberculosis therapy. Am J Respir Crit Care Med 2006; 174: 935-52.*

39. *Erdil A, Kadayifci A, Ateş Y, Bagci S, Uygun A, Dağalp K. Rifampicin test in the diagnosis of Gilbert's syndrome. Int J Clin Pract 2001; 55: 81-3.*

40. *Cascio A, Scarlata F, Giardano S, Antinori S, Colomba C, Titone L. Treatment of human brucellosis with rifampin plus minocycline. J Chemother 2003; 15: 248-52.*

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Effects of Saxagliptin on remission of patients with Type 2 Diabetes Mellitus

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Abstract

Objective: To study the effects of Saxagliptin on remission of patients with Type 2 diabetes mellitus (T2DM).

Methods: 101 patients with T2DM admitted in Endocrine Department of our hospital between November in 2010 and June in 2012 were selected as the study object, which were divided into two groups-Saxagliptin group (n = 52) and control group (n = 49) by matched pairs according to the course of disease, body mass index, fasting and postprandial C peptide, blood lipid and the original therapeutic scheme. The patients received 3 months of treatment, by once follow-up each month for the measurement of the levels of blood glucose, glycosylated hemoglobin (HbA1c), C-peptide and insulin, and 72 h continuous glucose monitoring and intravenous glucose tolerance test before treatment and three months after treatment respectively.

Results: After 3 months of treatment with Saxagliptin, 17.31% (n = 9) T2DM patients entered remission, which was statistically significant ($P < 0.05$) compared with that of the control group; the levels of fasting blood glucose, postprandial blood glucose and HbA_1c decreased after 3 months of Saxagliptin treatment compared with those before treatment, and the time rates of high blood glucose and low blood glucose dropped, with the difference statistically significant ($P < 0.05$).

Conclusion: The Saxagliptin treatment can promote patients with T2DM into remission.

Key words: Saxagliptin, T2DM, remission phase, treatment.

Introduction

Type 2 diabetes mellitus (T2DM) is generally considered as a non-curable disease, which requires a lifelong treatment [1]. In recent years, Chinese scholars have found that intensive insulin therapy can significantly improve the islet B cell function of newly-diagnosed T2DM patients so as to induce the occurrence of "remission" [2]. Glucagon-like Peptide-1 (GLP-1) is the body's main incretin, secreted by intestines after meals [3]. It can promote insulin secretion by the glucose-dependent way, inhibit glucagon secretion, delay gastric emptying and increase satiety, so as to maintain blood glucose stable [4]. GLP-1 can also improve the function of islet B cells in patients with T2DM. Saxagliptin is an efficient Dipeptidyl Peptidase 4 (DPP-4) inhibitor, which can increase the levels of endogenous GLP-1 [5] and glucose-dependent insulinotropic peptide (GIP) by selectively inhibiting DPP-4 so as to regulate blood glucose [6]. There is still a lack of relevant studies currently on whether Saxagliptin treatment can induce and prolong remission in T2DM patients.

In this study, 101 patients with T2DM admitted in our hospital between November in 2010 and June in 2012 were selected as the study object, which were divided into two groups-Saxagliptin group (n = 52) and control group (n = 49) by matched pairs according to the course of disease, body mass index, fasting postprandial C peptides, blood lipid and the original therapeutic scheme. After treatment, the proportion of patients in remission, blood glucose level and islet function were compared between the two groups to study the effects of Saxagliptin on remission in T2DM patients.

Materials and Methods

Inclusion Standards

The patients were diagnosed as T2DM according to the WHO's standard in 1999 [7].

Objects

101 patients with T2DM admitted in our hospital between November in 2010 and June in 2012 were selected as the study object.

Methods

The patients were divided into two groups: Saxagliptin group (experimental group) (n = 52) and control group (n = 49) by matched pairs according to the course of disease, BMI, fasting and postprandial C-peptides, blood lipid, the original therapeutic scheme for the study of the possibility of Saxagliptin treatment in inducing and prolonging remission. The research program was approved by the Hospital Ethics Committee of the hospital, and all the patients signed the informed consent before treatment.

Method of treatment

On the basis of maintaining the original therapeutic scheme (including 32 cases receiving subcutaneous insulin infusion therapy and 20 cases given oral hypoglycemic agents), the Saxagliptin group (experimental group) was given Saxagliptin tablets orally for 3 months, 4mg/d orally in the first month; 6mg/d in the second and third months. Saxagliptin treatment was stopped after 3 months. The therapeutic scheme remained the same as the original's in the control group (including 28 cases receiving subcutaneous insulin infusion therapy and 21 cases given hypoglycemic agents orally).

Reduction of insulin

The patients of the two groups conducted self-monitoring of blood glucose (SMBG) for 4 to 7 times daily, for example, where the levels of fasting blood glucose was less than 6.0 mmol / L, the insulin dose was reduced by 1 to 2 U, and made a record. As the insulin dose for the patients in the Saxagliptin group was less than 0.3 U/kg /d, they could stop being treated with insulin, and given Saxagliptin therapy only.

In the control group, as the insulin dose was less than 0.3 U/kg/d, insulin might be withdrawn, and switched to oral hypoglycemic agents according to the patients' preference; as the insulin dose was less than 6 to 10 U / d, the patients might stop using insulin, and control blood glucose by diet and exercises.

Follow-up method

The patients of the two groups were followed up for 1 month. Saxagliptin was withdrawn after 3 months of treatment, we continued to maintain therapy by reducing insulin dose or control blood glucose by diet and exercises. The patients were kept continued followed-up for 3 months after withdrawal, reviewed monthly for a total of 6 months. If the patients remained in remission three months after withdrawal, they continued to be observed by follow-up every month until the end of remission. During the follow-up period, the patients conducted SMBG for 4 to 7 times daily; as fasting blood glucose ≥ 7 mmol/L, or (and) postprandial glucose ≥ 10mmol/L, it indicated the end of remission, and appropriate hypoglycemic therapy was applied on the patients according to their willingness and blood glucose level.

Statistical analysis

All data were analyzed by SPSS 18.0 and expressed as x±s. $P<0.05$ was considered statistically significant.

Results

Baseline indicators comparison

The baseline indicators of the two groups did not differ significantly (Table 1).

Remission phase ratio comparison

The remission phase ratios of the two groups differed significantly ($P<0.05$) (Table 2).

Effects of Saxagliptin on blood glucose

FBG comparison

The FBG levels of the treatment group after 2 and 3 months of treatment were significantly lower than those before treatment ($P<0.05$), whereas those of the control group did not differ significantly ($P>0.05$) (Table 3).

Postprandial blood glucose comparison

The Postprandial blood glucose levels of the treatment group after 1 and 2 months of treatment were significantly lower than those before treatment ($P<0.05$), whereas those of the control group after 2 and 3 months and those before treatment did not differ significantly ($P>0.05$) (Table 4).

HbA_1c level

The HbA1c levels of the treatment group after 3 months of treatment were significantly lower than those before treatment ($P<0.05$), whereas those of the control group did not differ significantly ($P>0.05$) (Table 5).

Table 1. Comparison of baseline indicators between 2 groups (x±s)

Item	Treatment	Control
Age (years old)	44.24±11.39	45.12±11.59
Disease course (month)	24 (1～84)	20 (1～84)
BMI/kg.m^2	26.30±3.58	25.67±2.66
Waist/hip ratio	0.93±0.05	0.94±0.05
Systolic pressure (mmHg)	125.01±16.56	124.23±17.06
Diastolic pressure (mmHg)	81.02±8.78	80.79±9.14
FBG (mmol/L)	8.13±2.89	7.72±2.15
Postprandial glucose (mmol/L)	11.29±4.07	11.21±4.15
Fasting C peptide (ng/mL)	2.21±0.96	2.40±1.19
Postprandial C peptide (ng/mL)	4.19±2.54	4.93±3.09
HbA_1c (%)	10.09±2.67	9.43±2.18
TG (mmol/L)	2.17±1.54	3.52±3.55
TCHO (mmol/L)	4.89±0.96	4.80±1.35
HDL (mmol/L)	1.29±0.36	1.37±0.48
LDL (mmol/L)	2.11±0.68	2.10±0.59
CRP (m/L)	2.25±3.11	1.36±1.40
Insulin amount (U/kg/d)	0.24 (0～0.5)	0.23 (0～0.6)

Table 2. Comparison of remission phase ratio between 2group (s%)

	Treatment (n=52)	Control (n=49)
remission phase	17.31* (n=9)	0 (n=0)
2-month remission phase	3.85 (n=2)	0 (n=0)
3-month remission phase	5.77 (n=3)	0 (n=0)
6-month remission phase	7.69 (n=4)	0 (n=0)

*Compared to the control group: *P<0.05*

Table 3. Comparison of fasting blood glucose between 2group (smmol/L)

	Treatment	Control
Before treatment	8.13±2.89	7.72±2.15
Postoperative 1st month	7.43±1.29	7.12±1.51
Postoperative 2nd month	7.06±0.95*	6.97±0.25
Postoperative 3rd month	7.01±0.94*	6.61±0.89

*Compared to the results before treatment: *P<0.05*

Table 4. Comparison of postprandial blood glucose between 2 groups (mmol/L)

	Treatment	Control
Before treatment	11.29±4.07	11.21±4.15
Postoperative 1st month	7.94±1.65*	8.85±1.09*
Postoperative 2nd month	8.52±1.45*	9.06±1.37
Postoperative 3rd month	9.03±0.98	9.30±1.16

*Compared to the results before treatment: *P<0.05*

Table 5. Comparison of HbA1c (%) between 2 groups before grouping and after 3 months of treatment

	Treatment	Control
Before grouping	10.09	9.43
3 months of treatment	7.19*	7.65

*Compared to the results before grouping: *P<0.05*

Table 6. Comparison of blood glucose fluctuations between 2 groups

Item	Treatment (n=52)		Control (n=49)	
	Before	3 months	Before	3 months
Average blood glucose (mmol/L)	6.98±1.29	6.63±1.64	5.75±1.63	6.01±1.36
Blood glucose fluctuation coefficient	2.71±1.73	2.49±1.87	1.43±1.18	2.58±1.27
High-blood-glucose time ratio (%)	20.85±12.74	8.92±6.64*	11.9±4.52	10.25±2.64
Low-blood-glucose time ratio (%)	8.61±6.45	2.13±6.15*#	12.19±16.30	9.82±3.22
LAGE (mmol/L)	7.87±2.19	5.76±1.58	6.88±1.28	6.46±1.45
MAGE (mmol/L)	3.90±1.23	3.25±0.99	3.65±1.43	3.90±1.02
MODD (mmol/L)	1.50±0.73	1.43±0.91	1.52±0.47	2.07±0.65

*Compared to the results before grouping: *P<0.05; compared to the control group: #P<0.05*

Blood glucose fluctuation

The low-blood-glucose time ratio and the high-blood-glucose time ratio were reduced more significantly (P<0.05) after 3 months of treatment compared to those of the control group (Table 6).

Effects of Saxagliptin on insulin functions

The fasting C peptide levels of the treatment group were significantly elevated after 2 months of treatment (P<0.05) (Table 7). The postprandial C peptide levels of the treatment group were also significantly higher than those before treatment after 3 months of treatment (P<0.05) (Table 8).

Table 7. Changes of fasting C peptide between 2 groups (ng/mL)

Treatment time	Treatment	Control
Before	2.21±0.96	2.40±1.19
1 month	2.45±0.20	2.63±0.91
2 months	2.87±0.37*	3.12±1.13
3 months	2.76±0.31	3.44±0.93

*Compared to the results before treatment: *P<0.05*

Table 8. Changes of postprandial C peptide between 2 groups (ng/mL)

Treatment time	Treatment	Control
Before	4.19±2.54	4.93±3.09
1 month	4.14±1.87#	5.76±2.20
2 months	4.07±1.94#	5.78±1.46
3 months	4.41±1.04#*	6.16±1.98

*Compared to the results before treatment: *P<0.05; compared to the results of the control group: #P<0.05*

Discussion

GLP-1, secreted by small intestinal L cells, can promote insulin secretion in a glucose-dependent manner under the stimulation of nutrients [8]. 53% of amino acid sequences of GLP-1 are the same in Saxagliptin and human, therefore, it can bind with GLP-1 receptor to play the role similar to GLP-1 [9]. Saxagliptin is mainly used for the treatment of T2DM. At abroad, the majority of studies aim to patients with the disease course of five years or more, while there is rare clinical research domestically on the treatment of T2DM with Saxagliptin, and no study of Saxagliptin's effects on blood glucose fluctuation and remission [10]. Therefore, it is of great significance for this study to discuss the therapeutic effects of Saxagliptin on T2DM patients and remission.

Remission generally refers to the decrease in the amount of insulin, even no need of insulin in minority after 2 weeks to 3 months of insulin therapy for patients with Type 1 diabetes, which means to enter remission period [11]. Afterward, it is found that remission also exists in T2DM [12]. In 2008, the Chinese scholar Weng Jianping [13] found that intensive insulin therapy could significantly improve the islet B cell function of patients with T2DM. Blood glucose can be controlled well only by diet and exercises a few months to a year's time after the intensive treatment, which is entering the "honeymoon period" or "remission" similar to Type 1 diabetes [14]. The results of this study showed that 5 patients with T2DM could be withdrawn drug therapy 3 months after Saxagliptin treatment, suggesting that short-term Saxagliptin treatment can induce the occurrence of remission in T2DM patients, which brings new hope for the treatment of patients with

T2DM. However, its concrete mechanism is unclear yet, which may be relative with the improvement of islet B cell function or insulin resistance. After Saxagliptin treatment, the levels of fasting and postprandial blood glucoses and HbA1c were lower than those before treatment, indicating that Saxagliptin can reduce fasting and postprandial blood glucoses and HbA1c at the same time, which is consistent with overseas studies [15]. The improvement of islet B cell function contributes to the comprehensive control of blood glucose, and large blood glucose fluctuation itself is an independent predictor of mortality of diabetes [16]. Saxagliptin is able to promote insulin secretion in a glucose-dependent manner, thus effectively reducing the incidence of hypoglycemia [17], but there is no related research on its possibility of improving blood glucose fluctuation. Currently, the blood glucose measurement method frequently used clinically can only reflect a certain static instantaneous blood glucose level, while the duration of the state of night asymptomatic hypoglycemia and postprandial hyperglycemia are often unable to be accurately measured. In recent years, the technique of continuous glucose monitoring system (CGMS) has been gradually applied to clinical practice [18], which is capable of continuous monitoring of blood glucose for 72 h, glucose values recorded every 3 min. The CGMS can comprehensively understand the round-the-clock change information of blood glucose, provide the highest and lowest blood glucose levels in 24 h, so as to avoid missed diagnosis of unaware hypoglycemia and hyperglycemia, and accurately measure blood glucose fluctuation [19]. In this study, through CGMS monitoring on the blood glucose levels 72 h before and after Saxagliptin treatment, the results showed that the blood glucose fluctuation coefficient was on a declining trend, and the time rates of both hyperglycemia and hypoglycemia decreased after Saxagliptin therapy, which indicates that Saxagliptin is able to reduce the incidence of hypoglycemia, and may play a role in reducing blood glucose fluctuation pending further validation.

Islet B cell function is an important indicator to judge the progression of T2DM, but it is affected by the degree of blood glucose and insulin resistance and other factors [20]. After islet B cells synthesize proinsulin in vivo, then divided into insulin and C-peptide. Both release into the blood circulation with equal numbers, so the determination of the concentration of C-peptide in blood can understand the function of islet B-cell reserve. This study showed that fasting C-peptide level of patients with T2DM was higher after Saxagliptin treatment than before, but showed a downward trend compared with the control group; the postprandial C-peptide level was significantly lower than that of the control group, inconsistent with studies abroad. Bunck et al. [21] studied 36 T2DM patients with the mean disease duration of 5.7 years and BMI of 30.9 kg/m^2, and found that one-year Saxagliptin treatment could better control blood glucose, significantly improved various indicators of islet B cell function, in which 1-phase and 2-phase C-peptide secretion increased by (1.53 ± 0.11) and (2.85 ± 0.22) times, with statistically significant difference compared with that by insulin glargine ($P < 0.0001$); in addition, the release of arginine-stimulated C-peptide also increased by (3.19 ± 0.24) times; but the islet B cell function and glycemic level returned to the pretreatment levels 3 months after Saxagliptin withdrawal. It suggested that the effects of Saxagliptin on improvement of islet B cell function needed to be obtained from continued use.

In this study, the levels of fasting and postprandial C-peptides declined in the Saxagliptin treatment group compared with the control group. The reasons are considered that for the curative time of Saxagliptin is just three months, short-term treatment can only restore the secretion function of islet B cells inherent in the patients [22], and Saxagliptin has not yet had time to promote proliferation and differentiation of islet cells, whose main purpose is to improve the body's insulin resistance [23] so as to reduce endogenous insulin needed by the patients and save their own insulin secretion[24]; at the same time, the hypoglycemic effect of Saxagliptin is to promote insulin secretion in a glucose-dependent way. The blood glucose level decreased significantly in the patients, and even returned to normal level, after treatment, the effect of promoting insulin secretion also weakened consequently, so the results showed that the fasting and postprandial C-peptide levels declined [25].

This study confirms that Saxagliptin therapy can promote the occurrence of remission in T2DM patients, and improve blood glucose fluctuation and

insulin resistance. It is expected to become a reality to change continued treatment into intermittent treatment for T2DM, in order to bring a brand new approach for the treatment of patients with T2DM.

References

1. *Dekker D, Dorresteijn MJ, Pijnenburg M, Heemskerk S, Rasing-Hoogveld A, Burger DM, et al. The bilirubin-increasing drug atazanavir improves endothelial function in patients with type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol 2011; 31: 458-463.*

2. *Li YB, Zeng LY, Shi LX, Zhu DL, Zhou ZG, Yan L, et al. [The effects of early intensive therapy on islet beta cell function and long-term glycemia control in newly diagnosed type 2 diabetic patients with different fasting plasma glucose levels.]. Zhonghua Nei Ke Za Zhi 2010; 49: 9-13.*

3. *Speck M, Cho YM, Asadi A, Rubino F, Kieffer TJ. Duodenal-jejunal bypass protects GK rats from {beta}-cell loss and aggravation of hyperglycemia and increases enteroendocrine cells coexpressing GIP and GLP-1. Am J Physiol Endocrinol Metab 2011; 300: E923-932.*

4. *Christensen M, Vedtofte L, Holst JJ, Vilsboll T, Knop FK. Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in humans. Diabetes 2011; 60: 3103-3109.*

5. *Shubrook J, Colucci R, Guo A, Schwartz F. Saxagliptin: A Selective DPP-4 Inhibitor for the Treatment of Type 2 Diabetes Mellitus. Clin Med Insights Endocrinol Diabetes 2011; 4: 1-12.*

6. *Scheen AJ. [Saxagliptin (Onglyza): new inhibitor of the dipeptidylpeptidase-4 for the oral treatment of type 2 diabetes]. Rev Med Liege 2010; 65: 527-532.*

7. *Botas P, Delgado E, Castano G, Diaz de Grenu C, Prieto J, Diaz-Cadorniga FJ. Comparison of the diagnostic criteria for diabetes mellitus, WHO-1985, ADA-1997 and WHO-1999 in the adult population of Asturias (Spain). Diabet Med 2003; 20: 904-908.*

8. *Holst JJ, McGill MA. Potential new approaches to modifying intestinal GLP-1 secretion in patients with type 2 diabetes mellitus: focus on bile acid sequestrants. Clin Drug Investig 2012; 32: 1-14.*

9. *Wang A, Dorso C, Kopcho L, Locke G, Langish R, Harstad E, et al. Potency, selectivity and prolonged binding of saxagliptin to DPP4: maintenance of DPP4 inhibition by saxagliptin in vitro and ex vivo when compared to a rapidly-dissociating DPP4 inhibitor. BMC Pharmacol 2012; 12: 2.*

10. *Erhardt W, Bergenheim K, Duprat-Lomon I, McEwan P. Cost effectiveness of saxagliptin and metformin versus sulfonylurea and metformin in the treatment of type 2 diabetes mellitus in Germany: a Cardiff diabetes model analysis. Clin Drug Investig 2012; 32: 189-202.*

11. *Chen A, Huang Z, Wan X, Deng W, Wu J, Li L, et al. Attitudes toward diabetes affect maintenance of drug-free remission in patients with newly diagnosed type 2 diabetes after short-term continuous subcutaneous insulin infusion treatment. Diabetes Care 2012; 35: 474-481.*

12. *Pokala S. ACP Journal Club. Gastric bypass or biliopancreatic diversion increases remission from type 2 diabetes in obese adults. Ann Intern Med 2012; 157: JC2-12.*

13. *Weng J, Li Y, Xu W, et al. Effect of intensive insulin therapy on beta-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial[J]. Lancet 2008; 371: 1753.*

14. *Asma A, Azmi MN, Mazita A, Marina MB, Salina H, Norlaila M. A Single Blinded Randomized Controlled Study of the Effect of Conventional Oral Hypoglycemic Agents Versus Intensive Short-Term Insulin Therapy on Pure Tone Audiometry in Type II Diabetes Mellitus. Indian J Otolaryngol Head Neck Surg 2011; 63: 114-118.*

15. *Pfutzner A, Paz-Pacheco E, Allen E, Frederich R, Chen R. Initial combination therapy with saxagliptin and metformin provides sustained glycaemic control and is well tolerated for up to 76 weeks. Diabetes Obes Metab 2011; 13: 567-576.*

16. *Carroll AE, Marrero DG. The role of significant others in adolescent diabetes: a qualitative study. Diabetes Educ 2006; 32: 243-252.*

17. *Scheen AJ. Metformin + saxagliptin for type 2 diabetes. Expert Opin Pharmacother 2012; 13: 139-146.*

18. *Rimondi F, Banin P, Gamberini MR, De Sanctis V. The continuous glucose monitoring system (CGMS) in patients with beta-thalassemia major and impaired glucose homeostasis: preliminary results. Pediatr Endocrinol Rev 2008; 6 Suppl 1: 190-192.*

19. *Bilotta F, Rosa G. Glycemia management in critical care patients. World J Diabetes 2012; 3: 130-134.*

20. *He QH, Zhou YS, Wang Z, Wang S, Mou ZQ, Li J, et al. [Improvement of the function of islet beta and alpha cells by intervention against glucotoxicity: experiment with rats]. Zhonghua Yi Xue Za Zhi 2008; 88: 374-377.*

21. *Bunck MC, Diamant M, Cornér A, et al. One-year treatment with exenatide improves beta-cell function, com-pared with insulin glargine, in metformin-treated type 2 diabetic patients: a randomized, controlled trial[J]. Diabetes Care 2009; 32: 762.*

22. *Gautier JF, Sauvanet JP. Efficacy of saxagliptin as an add-on to oral monotherapy in the phase 3 clinical development program: predictive factors of the treatment response in type 2 diabetes. Ann Endocrinol (Paris) 2011; 72: 287-295.*

23. *Dave DJ. Saxagliptin: A dipeptidyl peptidase-4 inhibitor in the treatment of type 2 diabetes mellitus. J Pharmacol Pharmacother 2011; 2: 230-235.*

24. *Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, et al. The design and rationale of the saxagliptin assessment of vascular outcomes recorded in patients with diabetes mellitus-thrombolysis in myocardial infarction (SAVOR-TIMI) 53 study. Am Heart J 2011; 162: 818-825 e816.*

25. *Henry RR, Smith SR, Schwartz SL, Mudaliar SR, Deacon CF, Holst JJ, et al. Effects of saxagliptin on beta-cell stimulation and insulin secretion in patients with type 2 diabetes. Diabetes Obes Metab 2011; 13: 850-858.*

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A useful strategy for test anxiety treatment

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Abstract

The main purpose of this study was the effect of Education of self-regulation on test anxiety in boy students, The Society under study include all boy and girl students of shahrebabak branch. From this society 144 persons were randomly selected as sample that include experiment and control groups and 100 persons were selected for comparative analyzes.measurement tools was Test-anxiety inventory that encountered of reliability and validity.

Design research was of kind pre-test and post-test with control group. After of pre-test, experimental groups four week, every week, two sessions and every session, , an hour to share in education class of self-regulation. After of end experimental to accomplished post test with two groups. The results of T-test show that there are significant difference between test anxiety in pre-test and post-test. Also we can see differenced levels of test anxiety between this student (17.6=little, 52.9=average 19.6=high, 9.8 extreme). Mean scores of test anxiety among students whose parents have academic education is higher than whom their parents don't have academic education that show parents with academic education have more stringent whereas, results of t-test show that there are no significant differences between parents with academic education and no academic education also There are no significant different in mother profession and there are significant difference between boys and girls test anxiety ($t=-4.66$, $p<0.00$). With comparison Mean of two groups discover that girls encounter higher test anxiety.

Key words: Education, self-regulation, test anxiety, strategy, useful.

Introduction

One of worries and problems of educational system and students is exam problem. Exam, the word that all student are familiar with that and some of them worried by nearing its name of course anxiety have measure and severity and variation undulate. Severity of anxiety is when that exam is near and in exam night increased to its severity. For example terminal exam in may-June and spatially entrance exam. School dropout threaded student society. According to the educational and instructional ministry estimation (in every educational year) of every 100 student dependent to variety bases 45 person were failed and or experienced School dropout[1] According to the studies and empirical dates characterized that almost 60 present of failed students and or persons that showed educational recession in scores. In lower sections and grade were member students that have noticeable achievement however by passing the time and changing sections emerged changes in scores [1]

in the way [2] used of The Test Anxiety Inventory for Children and Adolescents (TAICA) test for assessment test anxiety that included scales (problems cognition and inattention, high performance, instrumental anxiety, high physiological arouse, social humiliation, worry and worry and lie). [3] assessmented two treatment that include regular desensitization and role playing for decreasing test anxiety. The result show that psychological role playing was very effective in anxiety treatment. Another study did by [4] The result showed that various factors effected on test anxiety and also consider that factors that increase student comprehension and resulted that cognition approach with relaxant technique and synthetic interventions decrease test anxiety and increase exam performance and Eye movement desensitization and reprocessing (EMDR) technique also decrease test anxiety and emotionals and worries chosen of this spatially [5] in the way [6] cognitive-behavior therapy (CBT) and acceptance-based behavior therapy (ABBT) compared for the treatment of test anxiety. Results showed that those receiving ABBT experiencing improvements in performance, whereas those receiving CBT exhibited reduced performance. In addition, there was a suggestion that ABBT might have been more

effective at reducing subjectively experienced test anxiety. there are inconsistent with [7] Cognitive and regular desensitization technique in decrease test anxiety were analyzed. Results showed that test anxiety was lower in both groups in comparison to control group but there was no significant difference between Cognitive and desensitization technique in lowering test anxiety. Another research was performed to evaluate the lowering methods of anxiety with the aim of training of study skills and cognitive–behaviour technique and combination of above mentioned technique and control group, results showed that in ternate groups test anxiety scores were lower than control group [8]. and behaviour therapy (concentrate on subjective skills) and cognition effect on test anxiety[9]. [10] tried to unravel the influence of various types of education on test anxiety levels that include a stressful, achievement-orientated education; a reassuring, task-orientated education; and an ambiguous education. It turns out that state anxiety and test anxiety do not increase more rapidly as a function of anxiety disposition under stressful conditions compared with reassuring conditions. As in previous research, stage-fright effects were observed in the sense that repeated measures of state anxiety and test anxiety showed a decline of average anxiety levels.

30 boys and girls participated in a 10 sessions group therapy that include training of muscle relaxation and dealing with stress, findings showed that in experimental groups test anxiety scores were lower than control group also it was higher in females in comparison to males. Group therapy had omnipotent influence in scores related to self-confidence [11] The evaluation of three methods namely cognitive therapy, training of reading skills and control group was done by [12] that showed significant different between control and main groups about test anxiety, The case study of Touki showed that the test anxiety lowered in cognitive therapy group in comparison to control group. Learning the skills of dealing with emotional in lowering test anxiety and enhancing self-confidence were very important. It is possible to education the abovementioned skills as life skills to prevent stress [13].

The current study by [14] examined whether material presented in an interactive treatment format was effective in reducing state anxiety and test anxiety and increasing academic performance. A wait-list control group served as the comparison group in this study. The interactive component of the program introduced the ability for participants to test their knowledge of each treatment component, to experience imaginal exposure, and to provide anxiety ratings during exposure sessions. It was predicted that the participants in the treatment group would experience a greater decline in test anxiety than the participants in the wait-list control group.

Also other assessment showed that courses and educational strategy provided by Newcastle university are better than medicinal and psycho treatment [15] effect educational in interest background and test anxiety assessed on 375 student. the result showed that wasn't significant relation but there was significant relation between practical educational with sexual and interest [16]. Furthermore, In educational low-motive student we faced with students that have high scores in class but by passing the time and changing study grades, their information forget and extinguish. They aren't able to use of their information in variety of positions. Teachers, also aren't able easily able to recognition this kind of students and eliminate their problems. Surfaces activities show that they are active in learning but evaluations don't support [17]. in a research that did by [18] resulted that exam time and reward in exam time can effect on test anxiety. so students divided into three groups. To first group many reward, second group average reward, third group little reward and result showed that the group that received average reward have lowest measure anxiety. Study achievement is need to use of rich learning guidelines and coequal self-esteem and relief of anxiety. Researcher doesn't view higher surface test anxiety the only factor of recession performance. But point to the other factors such as achievement motive decrease, the kind of achievement motive, insufficiency in way study, weak organization, analyze information, etc.

In this way researchers reported students that educated purifying, comprehension and the understanding of cognition aspects have less test anxiety than to groups that watched film about test anxiety and the group that had studied the reports to this syndrome [19]. And also in a research that did by [20] resulted that if students have skull duggery paper in test session their test anxiety decrease si-

gnificant. In other study metacognition role was considered as a metacognition on test anxiety and study theory. They studied Approaches and Study Skills Inventories for Students (ASSIST) Metacognitions Questionnaire (MCQ), and Test Anxiety Scale (TAS). The result showed that it is the metacognition that effected on test anxiety [21]. It seem that learning and study method effect on learning translation. Students that was trained by correct study method educated and used of appropriate study method have high study achievement. On the other way researcher reported that maximum students and even student-university experimented test anxiety that have negative result [22]. other important factors in making test anxiety, is that students are not familiar with correct study method and curriculum. Most of student study different texts with the same speed and method namely. They study mathematic like Persian.

Current research indicates that some gifted students possess better self-regulated learning strategies than their peers, however gifted students may have done very well in school without using good self-regulation strategies because of a combination of their high abilities and/or an unchallenging curriculum. If learning is relatively easy for someone, less effort, organization and other self-regulated activities are expended. Social conditions or personal issues may prevent students from developing self-regulated learning strategies. For some students who already have some of these strategies, social or personal issues may prevent them from using them regularly, and thus, they need to be helped and encouraged to do so. Some gifted and talented students display perfectionism and need to learn to strive for excellence (their personal best) rather than perfection. Some talented students with high potential may find it difficult to learn self-regulation when it is not taught, modeled, or rewarded by the adults in their home and family. Even if students interact regularly with adults who demonstrate self-regulation, they may fail to use these skills themselves due to peer pressure or refuse to use the strategies their parents or teachers regularly employ at home or school. Compared with low achieving students, high achievers set more specific learning goals, use a variety of learning strategies, self-monitor more often, and adapt their efforts more systematically. The quality and quantity of self-regulation processes is crucial. We must recognize that one self-regulation strategy will not work for all students, and that the use of only a few strategies will not work optimally for a person on all tasks or occasions. It is important that students learn to use multiple self-regulatory learning skills rather than single strategies. They must also learn that their goals and their choice of self-regulation strategies have to be continually adjusted. Our hope in this module is that we will be able to work with students to help them shift from performance goals to move towards mastery goals, focusing on understanding the material, persisting when they are challenged or their performance fails. This is especially critical for talented students who seldom experience high levels of challenge.

According to [23] self-regulated learning involves the regulation of three general aspects of academic learning.

First, self-regulation of behavior involves the active control of the various resources students have available to them, such as their time, their study environment (e.g., the place in which they study), and their use of others such as peers and faculty members to help them.

Second, self-regulation of motivation and affect involves controlling and changing motivational beliefs such as self-efficacy and goal orientation, so that students can adapt to the demands of a course. In addition, students can learn how to control their emotions and affect (such as anxiety) in ways that improve their learning.

Third and finally, self-regulation of cognition involves the control of various cognitive strategies for learning, such as the use of deep processing strategies that result in better learning and performance than students showed previously

How does self-regulation develop?

Emotional self-regulation and cognitive self-regulation seem to have the same neural roots; thus, as children grow older and their brains develop, they can increasingly take control of both their thinking and their feelings. Furthermore, if a neural system is repeatedly exercised, it will continue to develop, as with exercising a muscle. Conversely, if children do not systematically engage in self-regulatory behaviors at a young age, the corresponding brain areas may not develop to their full potential [24], [25].

Before of this research, there was not any report of effect training self-regulation on test anxiety and in several research were assessed only the test anxiety relation with variables as fear of success, ways to nurture child, personality pattern, introversion-extraversion and the effect of different treatment and also was assessed purpose orientations with learning methods and test anxiety. According to this theory is it possible self-regulation effect on test anxiety? Yet to response to this question a research did on the boy student of third level of high school in shahrbabak.

Method research

With due attention to this self-regulation suppose as independent variable that researcher supervise in this and also existence of control group is experimental. It means made that tried with making independent variable that include self-regulation, consequences in dependent variable namely assess test anxiety, academic achievement and metacognition in selected students. Design research was of kind pre-test and post-test with control group.

The Society under study include all junior boy and girl students of shahrebabak branch. From this society 144 persons selected as sample randomly that put in experiment and control groups that.(100 persons were boy students of third level of high school and 44 persons were boy students in guidance school). and 100 persons selected for comparative analyzes.

Design research was of kind pre-test and post-test with control group. For administrating research test anxiety, academic achievement and meatcognition questionnaires administered for two control and experiment group as pre-test. Then all students of experiment group sat encounter in instruction methods training by 8 section, every section, an hour with use of participatory learning and techniques such as lecture, disputation and question and answer and then post-test do. Provided material in sections were:

First section: in the section produced description of effective study and qualification educated that learned to person how of study times have high use.

Second section: the principles of programming, suitable nurture, time of study trained

Third section: it trains about this why we should review subject and also how review them

Fourth section: in this section learning technique one time for always was trained that mastermind by Robinson in year 1996 and its name SQ3R. In the way try to make background in mind before of study, learning process become more and easy

Fifth section: one of the most important learning skills was comprehension and for this need to motive, concentration, pre background and correct study method. In the background given necessary recommendations specially in background note-writing and memory-tree to students

Sixth section: technique given for increasing mind activity and concentration

Seventh section: fast reading and its obstacles and necessary explanations given to students

Eighth section: necessary explanations given for better performance in exam.

Measurment tool

1- test anxiety questionnaire(TAL)include of 25 item that respond according to four division scale(0=never, 1=seldom, 2=somewhen, 3=often) minimum and maximum score in the test is zero, 75 respectively and also the test encounter suitable reliability and validity.

Internal consistency: for assessment Internal consistency (TAL) used of Alph Kronbakh. According to provided results Alpha coefficient was for all sample girl and boy, 0.94, 0.95, 0.92 respectively

Retest validity: for assessing validity scale TAL the test repeat afterward from 4 week to 6 week for 91 boys and 90 girls that participate in first stage. Mean and standard deviation of total score girl and boy persons in scale (TAL) in retest stage was y=34/24 (sd=17/26), X=32/28 (sd=15/8), x=2/36 (sd=19/44) respectively. Correlation coefficient between scores of persons in two stage test and retest for all persons include girl and boy were (r=0/77), (r=0/88), (r=0/67) that is satisfactory.

Reliability: for reliability evaluation, TAL this scale with anxiety questionnaire and scale self-steam given to sample students of research to do reliability

Scale 20 item anxiety questionnaire make by factor analysis method and encounter of acceptable and satisfactory psychometric characteristics

was made. Correlation coefficient between all person scores in general anxiety scale or TAL for all sample persons girl and boy are r=0/67, r=0/72 (p=0/001) respectively

Also for reliability assessment TAL used of self-steam Kooper Esmite scale that have 58 division and encounter of satisfactory reliability and validity. Correlation coefficient of all persons scores, girl and boy were in scale self-steam with TAL(r=0/57, r=0/67, r=0/43), (p=0/001) respectively. Researcher also accounted norms scores of sample persons (N=581) as percent grade and resulted with comparison person scores girl and boy in scale TAL by one test T. in the result girl scores were more than boys in scale TAL(T=3/31, df=524, p<0/0001)at the end, researcher resulted that TAL scale is bonafide for using in psychology researches and recognition test anxiety in children and adolescents in schools [26].

Results

In the research data analysis with description and inferential statistical method instance mean, standard deviation and t test

Test anxiety questionnaire (TAL) include 25 item that responds in basis one scale four division (0=never, 1=seldom, 2=somewhat, 3=often)minimum and maximum score in the test is zero, 75 respectively.

This test perform in 244 persons, society of boy in shahrbabak city and maximum score test anxiety was 66 and so students were ranking .

Test anxiety analyzes:

Table 1. Percent, median, mean students to severance degrees test anxiety

Descriptive statistical				
Degree	**mean**	**median**	**number**	**percent**
1	15.11	17	9	17.6
2	28.66	40	27	52.9
3	40.90	49	10	19.6
4	56.80	55	5	9.8
total	31.43	30	51	100

The results of table 1 show that percentages of student have test anxiety low, average, much, very much. 17.6, 52.9, 19.6, 9.8 respectively.

Demographic table shows that fathers and mothers of 43 and 13 percentage of students have academic education.

The table shows that the mean score of test anxiety among students whose parents have academic education is higher that show parents with academic education have more stringent. However there is no significant different in mother profession.

As it show in table 4 the mean scores of test anxiety change from 31.43 to 22.90 that showed self-regulation is useful and about control group did not change but improved certainly by approaching tests.

Table 2. Demographic table

Groups	**Education of father**		**Education of mother**		**mother 's job**	
	Acade	**No. A**	**Acade**	**No A**	**House wife**	**employee**
percent	43.1	56.9	13.7	86.3	74.5	25.5

Acade: academic *No. A: no academic*

Table 3. Descriptive statistical to compare parents education and profession

group		**mean**	**median**	**St.deva**	**kurtosis**	**Skew**
Edu of M	acad	37.71	30.00	12.26	0.72	0.91
	No.a	30.43	30.00	11.53	0.32	0.36
Edu of F	acadr	33.63	33.50	14.35	0.10	0.28
	No.a	29.75	30.00	10.36	1.46	0.73
Mother's p	House wife	31.13	30.00	12.49	-0.18	0.28
	employee	32.30	31.00	12.02	5.16	1.80

Edu of M: education of mother, Edu of F: education of father
Mother's p: mother's profession, No. A: no academic
Acade: academic

Table 4. Number, mean, std-deviation in pre-test and post-test

Groups	number		mean		std-deviation	
	Exper.	control	Exper.	control	Exper.	control
Pre-test	50	50	31.43	32.48	12.42	12.42
Post-test	50	50	22.90	34.68	7	14.57

Expe: experimental

Table 5. T-test for comparison pre-test and post-test

Groups	Mean	T	df	sig
Pre-test	31.43	7.79	50	0.00
Post-test	22.90			

Table 5 show that self-regulation can effect in test anxiety (t=7.79, p<0.000).

Table 6. Comparison test anxiety in boy and girl students

Groups	Mean	T	df	sig
Boy	31.43	-4.66	50	0.00
girl	41.37			

Upper table show that there are significant different between boys and girls test anxiety score (t=-4.66, p<0.00) and we find by comparing mean of two groups that girls encounter higher test anxiety.

Table 7 shows that there are no significant differences between two groups based on father's education.

Table 8 shows that there are no significant differences between two groups based on mother's education.

Conclusion

Students that have test anxiety although they learned subjects and concepts and course subjects are not able to provide and express self taught. Society and spatially education, of is fond and worry toward destiny of student in relation with this problem and successful development evolution in society and it expected that should achieve required abilities and skills and it expected that students should achieve required abilities and skills in different cognition, affective, personality aspects to achieve development and ascendancy on the other hand should recognize factors that caused better education and efflorescence of student capacities, and after given them achievement and development.

There is a growing awareness among developmental scientists that the better a child can self-regulate, the better she can rise to the challenge of mastering ever more complex skills and concepts. In the simplest terms, self-regulation can be defined as the ability to stay calmly focused and alert, which often involves – but cannot be reduced to – self-control. The better a child can stay calmly focused and alert, the better he integrates the diverse information coming in from his different senses, assimilates it, and sequences his thoughts and actions. For someone who thinks that self-regulation is really just a matter of a child's

Table 7. Comparison of test anxiety based on father's education

Test anx. sc.	Levene's T. for Equ. of Var.		t.test for Equ. of M.		
	F	sig	t	df	Sig. (2-ta)
Equ.var. ass	3.10	0.08	-1.12	49	0.26
Equ. Var. not ass			-1.07	36.60	0.29

Equ. var. ass: equal variance assumed, M: mean

Table 8. Comparison of test anxiety based on mother's education

Test anxiety sc.	Levene's T for Equ of Var		t.test for equ of M		
	F	sig	t	df	Sig.(2.ta)
Equ. var. ass	0.77	0.38	-1.47	49	0.14
Equ.var. not as.			-1.17	7.06	0.27

getting in control of his negative emotions, there is very little difference between self-regulation and compliance. But, unlike compliance based on punishment, self-regulation nurtures the ability to cope with greater and greater challenges because it involves arousal states, emotions, behavior, and – as the child grows older – thinking skills.

The results of T-test show that there are significant difference between test anxiety in pre-test and post-test. Also we can see differenced levels of test anxiety between this student (17.6=little, 52.9=average, 19.6=high, 9.8 extreme). Mean scores of test anxiety among students whose parents have academic education is higher than whom their parents don't have academic education that show parents with academic education have more stringent whereas results of t-test show that there are no significant differences between parents with academic education and no academic education also There are no significant different in mother profession.

Also there are significant difference between boys and girls test anxiety ($t=-4.66$, $p<0.00$). With comparison Mean of two groups discover that girls encounter higher test anxiety. [28]

Comprehend students that have infirmity in information analysis and subject organization experience higher test anxiety during exam. Students that used of low learning level method have problem in information analysis and profound understanding of lesson subjects and result that caused weak academic performance and experience high test anxiety.

Self-regulation is a deep, internal mechanism that enables children as well as adults to engage in mindful, intentional, and thoughtful behaviors. Self-regulation has two sides:

first, it involves the ability to control one's impulses and to stop doing something, if needed—for example; a child can resist his immediate inclination to blurt out the answer when the teacher poses a question to another child.

Second, self-regulation involves the capacity to do something (even if one doesn't want to do it) because it is needed, such as awaiting one's turn or raising one's hand. Self-regulated children can delay gratification and suppress their immediate impulses enough to think ahead to the possible consequences of their action or to consider alternative actions that would be more appropriate. While most children know that they are supposed to "use their words" instead of fighting, only children who have acquired a level of self-regulation are actually able to use them. This ability to both inhibit one behavior and engage in a particular behavior on demand is a skill used not just in social interactions (emotional self-regulation) but in thinking (cognitive self-regulation) as well. For example, to read the word cat when it appears under a picture of a dog, a child must overcome the desire to pay more attention to the picture and instead focus on the word [29]. In fact, research shows that children's self regulation behaviors in the early years predict their school achievement in reading and mathematics better than their IQ scores [30], [31] are almost similar this research, results of academic achievement analyzes show mean scores of courses increase in post-test that show self-regulation is useful in practicable and reading courses. Also The results this research is the same as [32] that showed student that used of higher learning method experience more academic achievement than students that used of low learning methods. [33] in every two self study discovered significant and positive relation between academic achievement and profound methods. In research [34] mind review method negatively and supervision in subject perception positively were predictor of academic achievement. And also results of [35] showed that education of cognitive method and met cognitive and cooperative study cause students exhibit better performance in course scores and self-regulation process can effect on decreasing test anxiety students [36]. self-regulation, self-esteem know as negative predictor of test anxiety and external motivations as predictor positive test anxiety [37]. These findings suggest that the Head-to-Toes Task(HTT) may be a useful measure of behavioral regulation for Taiwanese preschoolers and provide evidence for the importance of behavioral regulation for academic achievement in Taiwan. Practical implications focus on supporting the development of behavioral regulation in early childhood settings, which can promote early school success [38]. The present study [39] examined the efficacy of a self-regulation intervention with 65 preschool children. Using circle time games, the study examined whether participating in a treatment group significantly improved behavioral self-regulation and early academic outcomes. The

findings from this study provide preliminary evidence for the efficacy of the intervention in terms of improving preschoolers' behavioral self-regulation for children low in these skills and improving letter-word identification. Although preliminary, these results have the potential to inform preschool curricula that emphasize behavioral self-regulation as a means of facilitating school readiness. findings suggest that students' self-regulation of learning, self-efficacy beliefs, academic delay gratification, and final course grade are related [40]. Results [41] obtained are mirroring perceived competence of self-regulation and differ from the results concerning metacognitive accuracy. Metacognitive self-regulation persists as an important predictor of school achievement at all developmental levels, and the motivational self-regulation has significant impact on performance in the first and second age group. Finally, children's chronological age, and not whether they experienced one versus two years of preschool, predicted children's vocabulary and self-regulation outcomes. Implications for preschool curricula and instruction are discussed, including the increasing emphasis on literacy learning prior to kindergarten entry and the need to address self-regulation development along with academic learning [42]. Analyses [43] also suggest significant benefits of Chicago School Readiness Project (CSRP) for children's preacademic skills, as measured by vocabulary, letter-naming, and math skills. Partial support was found for improvement in children's self-regulation as a hypothesized mediator for children's gains in academic readiness. Implications for programs and policies that support young children's behavioral health and academic success.

Students that encounter in evaluation and exam continuously have low test anxiety and high achievement motive[44] that in this way [45] discovered that students who experience test anxiety in high level when exams them online test anxiety decrease and obvious natural abilities. The current study by [46] influence of genetical evaluation on lowering test anxiety and students academic achievement were analyzed and results showed that they ameliorated the above-mentioned skills but they had no influence on students creativity. Findings [47] provide partial support for the self-regulatory model of test anxiety. suggesting that additional routes are required to account for the role of parental pressure and teachers' performance-avoidance goals and a re-examination of the relationship between test anxiety and achievement goals. Factor analysis of responses to survey items by a second sample of students refined the initial framework. The final framework comprised three broad domains of perceptions of sources of test anxiety: students' perceptions of the test, their self-perceptions, and their perceptions of the test-taking situation [48]. other research [49] focuses on the interaction between students' domain-specific expectancies and values as a predictor of test anxiety. Those students who highly value success in math or English yet expect to do poorly in those subjects report the highest levels of test anxiety. Effect sizes are larger for math than English. Few gender differences emerge, but one prospective analysis reveals that girls who devalue English are more likely to maintain moderate levels of test anxiety across the transition to junior high school. also [50] investigated self-efficacy, gender and trait anxiety as moderators of test anxiety. Results of regression analysis indicated that the model was significant. Self-efficacy contributed 14% of the variability in test anxiety, whereas trait anxiety moderated 49% of the variability in test anxiety. Gender was not a significant predictor of test anxiety. Persons with lower self-efficacy had higher test anxiety scores. Also There are Negative correlations between deductive reasoning and standardized test scores [51].

Results [52] make clear this that test anxiety have significant and positive relation with past exam experience and significant and inverse relation with self-steam. [53] selected number 2482 person in self research and discovered that a) there are weak relation between test anxiety and achievement b)there are positive and significant relation between self-concept and study habits with achievement.

Suggestions

In the end with regard to results of this study self-regulation education can have a effective role in students test anxiety. So it necessary about this problem more serious. And make courses as the way of study for students to decrease this problem and also achievement and society bright capacity growing.

How help to Self-Regulate in Children?

1 Model self-control and self-regulation in your words and actions when you are frustrated with a classroom situation.
2 Provide structure and predictability. Children with self-regulation problems are internally "unstructured." The more freedom and flexibility they have, the more likely they are to demonstrate uncontrolled behaviors.
3 Anticipate transitions and announce changes in classroom schedules.
4 Reward children with good self-regulation capabilities with freedom and flexibility that will offer them opportunities for spontaneous, creative play and learning.
5 Try to identify the most "reactive" and impulsive children and keep them apart from each other. Pairing children who face these challenges can escalate the problem.
6 Remember that impulsive and aggressive children can create an atmosphere of chaos and fear that inhibit the capacity of other children to learn. Don't be afraid to immediately re-direct inappropriate words and actions. Your actions will make the rest of the children feel safer.
7 Seek help. Don't be afraid to point out a child's self-regulation problems with parents or other school personnel. Early identification and intervention can save the child and family years of failure and pain [54].

References

1. *Spalding L. Motivation in lesson class. Trans. Yaghoobi, Hasan. Hoshkholgh, Eraj. publications university teacher training . Tabriz, 1997*

2. *Whitaker S, Jolyn D, Lowe PA, Lee SW. "Significant Predictors of Test Anxiety among Students with and without Learning Disabilities" Journal of Learning Disabilities, 2007; v40 n4: p360-376.*

3. *Kipper DA, Giladi D. "Effectiveness of Structured Psychodrama and Systematic Desensitization in Reducing Test Anxiety". Journal of Counseling Psychology, 1978; v25 n6: 499-505.*

4. *Gregor A. "Examination Anxiety: Live with It, Control It or Make It Work for You?". School Psychology International, v26 n5 p617-635. 2005.*

5. *Enright M. Baldo TD. Wykes SD. "The Efficacy of Eye Movement Desensitization and Reprocessing Therapy Technique in the Treatment of Test Anxiety of College Students". Journal of College Counseling, 2000; v3 n1: 36-48.*

6. *Brown LA, Forman EM, Herbert JD, Hoffman KL, Yuen EK, Goetter EM. A Randomized Controlled Trial of Acceptance-Based Behavior Therapy and Cognitive Therapy for Test Anxiety: A Pilot Study. Behavior Modification, 2010; v35 n1: 31-53.*

7. *Fathi AA, Imam GVF. The comparision of cognitive therapy methods and methodical desensitization in lowering test anxiety kousar medical journal. 2002*

8. *Amiri B, Ghanbari HA, Bahram A, Agha MSH. The comparison of test anxiety lowering on a group of pre-university adolescent. Psychology and clinical psychiatry review. 2005*

9. *Ergene T. Effective Interventions on Test Anxiety Reduction: A Meta -Analysis. (EJ672677) School Psychology International, 2003; v24 n3: 313-28.*

10. *Meijer J, Oostdam R. Effects of Instruction and Stage-Fright on Intelligence Testing. European Journal of Psychology of Education, v26 n1: 143-161*

11. *Bahrehdar HH, Gafar M, Zahra RS. The effectiveness of test anxiety lowering on a group of pre-university adolescents. Psychology and clinical psychiatry review. 2009*

12. *Sobhi GN. Rasoulzadeh TK, Azad FP, Evaluation of effectiveness of cognitive therapy and instructing reading skills on students suffering from test anxiety. Tabriz university psychology journal. 2008*

13. *Pashaie Z. Pour Ebrahimi, Taghie. Khosh Konesh, Abolghasem. The influence of skills trained to deal with thrills of stress and education performance of high school. 2009*

14. *Pless A. Treatment of Test Anxiety: A Computerized Approach. ProQuest LLC, Ph.D. Dissertation, Central Michigan University. 2010*

15. *Feletti Grahame I, Neame Roderick LB. "Curricular Strateiges for Reducing Examination Anxiety" Higher Education, 1981; v10 n6: 675-86.*

16. *Schaffner E, Schiefele U. The Effect of Experimental Manipulation of Student Motivation on the Situational Representation of. Learning and Instruction, 2007; v17 n6: 755-772.*

17. *Jafari Majid. Assessment relation achievement motive and learning ways with test anxiety in boy guidance students in third degree. Thesis master science. Tehran university. College psychology and instructional science. 1997.*

18. *Chamorro-Premuzic T, Ahmetoglu G, Furnham A. Little More than Personality: Dispositional Determinants of Test Anxiety (the Big Five, Core Self-Evaluations, and Self-Assessed Intelligence) Learning and Individual Differences, 2008; v18 n2: 258-263.*

19. *Passig D, Moshe R. "Enhancing Pre-Service Teachers' Awareness to Pupils' Test-Anxiety with 3D Immersive Simulation", Computing Research, 2008; v38 n3: 255-278.*

20. *Erbe B. "Reducing Test Anxiety while Increasing Learning: The Cheat Sheet" College Teaching, 2007; v55 n3: 96-98.*

21. *Spada MM, Nikcevic AV, Moneta GB, Ireson J. Metacognition as a Mediator of the Effect of Test Anxiety on a Surface Approach to Studying. Educational Psychology, Oct 2006; v26 n5: 615-624.*

22. *Weiner B. History of Motivatonal Reasearch In Educational; Journal of Educational Psychology. 1990; 82(4): 616-622.*

23. *Pintrich PR, Smith DAF, Garcia T, McKeachie WJ. Reliability and predictive validity of the Motivated Strategies for Learning Questionnaire (MSLQ). Educational and Psychological Measurement, 1993; 53: 801-803.*

24. *Blair C, Razza RP. Relating effortful control, executive function, and false belief understanding to emerging math and literacy ability in kindergarten. Child Development, 2007; 78 (2): 647–63.*

25. *Diamond A, Barnett WS, Thomas J, Munro S. Preschool program improves cognitive control. Science, 2007; 318 (5855): 1387–88.*

26. *Biabangard E. Test anxiety. Tehran. Eslamic culture publication. 1998 Mokhtari, Richard. The meta cognitive awareness of reading strategies inventory(MARSI). Translate by navidi, ahad. Publish in Sina behavior science. 2002*

27. *Naveh-Benjamin M, Lin YG. Two types of test-anxious students: Support for and information processing model. Journal of Educational Psychology, 1987; 131-136.*

28. *Bialystok E, Martin MM. Notation to symbol: Development of a child's understanding of print. Journal of Experimental Child Psychology. 2003; 86: 223–43.*

29. *Blair C. School readiness: Integrating cognition and emotion in a neurobiological conceptualization of children's functioning at school entry. American Psychologist, 2002; 57 (2): 111–27.*

30. *Blair C, & Razza RP. Relating effortful control, executive function, and false belief understanding to emerging math and literacy ability in kindergarten. Child Development, 2007; 78(2): 647–63.*

31. *Valimohammadi MD. Assessment goal orientation and the learning strategis and test anxiety in second student of guidance in tehran city. 2001*

32. *Miller RB, Behrens JT, Greene BA & Newman DE."Goals and perceived Ability: Impact on stuendent valuing self-Regulation and persistence". Contemporary Educational psychology 18 2.14. 1993.*

33. *Basri A. Assessment comparative learning ways in student normal and prominent. Thesis master science, Tehran university. 1995*

34. *Enaiate M, Heidare A, Shahvali G. The effect of study training by cognitive strategies, and cooperative script method on academic performance of second grade male guidance school in dezful. Journal new findings in psychology. Propagations Ahvaz university. 2005*

35. *Parks-Stamm EJ, Gollwitzer PM, Oettingen G. Implementation Intentions and Test Anxiety: Shielding Academic Performance from Distraction. Learning and Individual Differences, 2010, v20 n1; 30-33.*

36. *Bembenutty H. Test Anxiety and Academic Delay of Gratification. College Student Journal, 2011; v43 n1: 10-21*

37. *Wanless SB, McClelland MM, Acock AC, Chen, Fu-Mei; Chen Jo-Lin. Behavioral Regulation and Early Academic Achievement in Taiwan. Early Education and Development. 2011; v22 n1: 1-28*

38. *Tominey, Shauna L.; McClelland, Megan M. Red Light, Purple Light: Findings from a Randomized Trial Using Circle Time Games to Improve Behavioral Self-Regulation in Preschool. Early Education and Development, 2011; v22 n3: 489-519.*

39. *Di Benedetto KM, Bembenutty H. Within the Pipeline: Self-Regulated Learning and Academic Achievement among College Students in Science Courses. Online Submission, Paper presented at the Annual Meeting of the American Educational Research Association (New Orleans, LA, Apr 8-12, 2011)*

40. *Bakracevic Vukman K, Licardo M. How Cognitive, Metacognitive, Motivational and Emotional Self-Regulation Influence School Performance in Adolescence and Early Adulthood. Educational Studies, Jul 2010; v36 n3: p259-268.*

41. *Skibbe LE, McDonald CC, Morrison JF, Jewkes MA. Schooling Effects on Preschoolers' Self-Regulation, Early Literacy, and Language Growth. Early Childhood Research Quarterly, 2011, v26 n1, 42-49.*

42. *Raver CC, Jones SM, Li-Grining C, Zhai F, Bub K, Pressler E. CSRP's Impact on Low-Income Preschoolers' Preacademic Skills: Self-Regulation as a Mediating Mechanism. Child Development, Jan-Feb 2011; v82 n1: 362-378.*

43. *Hancock DR. Effects of Test Anxiety and Evaluative Threat on Students' Achievement and Motivation. Journal of Educational Research, 2001; v94 n5: 284-90.*

44. *Stowell JR, Bennett D. Effects of Online Testing on Student Exam Performance and Test Anxiety. Journal of Educational Computing Research, 2010; v42 n2: 161-171.*

45. *Nemati S, Zahra S. Valaie N. Phani, hojat. influence of genetical evaluation on lowering test anxiety and students academic achievement in marvdasht city. Research method in dentistry science. 2009*

46. *Putwain WD, Woods AK, Symes W. Personal and Situational Predictors of Test Anxiety of Students in Post-Compulsory Education. British Journal of Educational Psychology, 2010; v80 n1: 137-160.*

47. *Bonaccio S, Reeve LC. The Nature and Relative Importance of Students' Perceptions of the Sources of Test Anxiety. Learning and Individual Differences, 2010; v20 n6: 617-625.*

48. *Selkirk CL, Bouchey AH, Eccles SJ. Interactions among Domain-Specific Expectancies, Values, and Gender: Predictors of Test Anxiety during Early Adolescence. Journal of Early Adolescence, 2011; v31 n3: 361-389.*

49. *Onyeizugbo UE. Self-Efficacy, Gender and Trait Anxiety as Moderators of Test Anxiety. Electronic Journal of Research in Educational Psychology, 2010; v8 n1: 299-312.*

50. *Rich DJ Jr., Fullard W, Overton Willis. The Relationship between Deductive Reasoning Ability, Test Anxiety, and Standardized Test Scores in a Latino Sample. Hispanic Journal of Behavioral Sciences, 2011; v33 n2: 261-277.*

51. *Capa Y, Loadman WE. "self-Efficacy and Previous Testing Experiences as Predictors of Test Anxiety among College Students". 2001*

52. *Mohan PR, Abebech A. Recalled Test Anxiety in Relation to Achievement, in the Context of General Academic Self-Concept, Study Habits, Parental Involvement and Socio-Economic Status among Grade 6 Ethiopian Students Education 3-13. Aug 2009; v37 n3: 269-285.*

53. *Brucy P. Self-Regulation: The Second Core Strength. Early childhood today. www. ChildTrauma.org*

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Application of ultrasonic scalpel in open thyroid operation with small incision and its effect on the quality of life of patients

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Abstract

Objective: To investigate the clinical value of ultrasonic scalpel in open thyroid operation with small incision and its effect on the quality of life of patients.

Methods: One hundred and ninety-six patients were randomly divided into an observation group and a control group. The patients in the observation group were given ultrasonic scalpel open thyroid operation with small incision, while those in the control group were given traditional electrosurgical knife operation. The indices of total operation time, thyroid resection time, intraoperative blood loss, postoperative drainage volume and complications, and quality of life were compared between the two groups.

Results: The total operation time, thyroid resection time, intraoperative blood loss and postoperative drainage volume in the observation group were significantly lower than those in the control group (P 0.05). The incidence of postoperative complications showed no significant difference between the two groups (P>0.05). The quality of life score (6.31±3.24) in the observation group was significantly less than that (13.56 ± 4.92) in the control group (P<0.05).

Conclusion: Ultrasonic scalpel application in open thyroid operation with small incision has advantages of short operation time, less blood loss and postoperative drainage volume, and better quality of life. It is worthy of further clinical application.

Key words: Ultrasound knife, traditional electrosurgical knife, small incision for thyroid operation, quality of life.

Introduction

Thyroid diseases are common in general surgery, including metabolic, functional, neoplastic, and inflammatory diseases. Several complications will occur if the patients are not treated in time[1-3]. Currently, the incidence of thyroid diseases has been increasing, and its composition also varied widely. Along with the development of modern medicine, surgery plays an increasingly important role in the treatment of thyroid diseases, and the surgical procedures are different according to different diseases. The ultrasonic scalpel is developed in recent years, which can cut body tissues with its strong penetrating power. Besides, the ultrasonic scalpel can be used for stopping bleeding and non-muscle electrostimulation with the advantages of less smoke and thermal damage. The technique was firstly used in laparoscopy, however, the ultrasonic scalpel become widely used in open thyroid surgery in recent years. According to other literatures, the ultrasonic scalpel applied in small incision thyroid surgery can significantly reduce operation time and intraoperative blood loss as well as the incidence of postoperative complications[4-7]. In our study, we compared the clinical application of ultrasonic scalpel and traditional electrosurgical knife in small incision thyroid surgery, and discussed their clinical efficacy and impact on quality of life in patients with thyroid diseases.

Material and Methods

General Information

One hundred and ninety-six patients with thyroid disease in our hospital from June 2009 to June 2011 were enrolled and randomly divided into an observation group and a control group (n=98). There were 32 male patients and 66 female patients with an average age of (40.56±4.06) years old in the observation group, including thyroidectomy + bilateral cervical lymph node dissection in 5 patients, thyroidectomy + unilateral cervical lymph

node dissection in 6 patients, thyroid lateral lobe + isthmus + contralateral lobe major resection in 15 patients, thyroid lateral lobe + isthmus resection in 21 patients, and thyroidectomy in 51 patients. Postoperative pathology suggested thyroid follicular carcinoma in 2 patients, toxic nodular goiter in 8 patients, Hashimoto's thyroiditis in 8 patients, thyroid papillary carcinoma in 21 patients, and nodular goiter in 59 patients. There were 30 male patients and 68 female patients with an average age of (38.78±3.72) years old, including thyroidectomy + bilateral cervical lymph node dissection in 6 patients, thyroidectomy+unilateral cervical lymph node dissection in 7 patients, thyroid lateral lobe+ isthmus + contralateral lobe major resection in 14 patients, thyroid lateral lobe + isthmus resection in 19 patients, and thyroidectomy in 52 patients. Postoperative pathology suggested thyroid follicular carcinoma in 3 patients, toxic nodular goiter in 7 patients, Hashimoto's thyroiditis in 9 patients, papillary thyroid carcinoma in 19 cases, and nodular goiter in 60 patients. The age, sex and disease composition between the two groups had no significant difference (P>0.05), and the data was comparable.

Treatment

The patients in the observation group were given ultrasonic scalpel open thyroid operation with small incision, and the patients in the control group were given traditional electrosurgical knife operation. The surgery of the two groups of patients was performed by same surgeon, and the relevant steps referred to our "standard operating procedure of ultrasonic scalpel open thyroid operation with small incision" and "standard operating procedure of traditional electrosurgical knife open thyroid operation with small incision".

Observation indices

Total operation time, thyroid resection time, intraoperative blood loss, postoperative drainage volume and complications, and quality of life were compared between the two groups. Quality of life scoring referred to DLQI scale[3]. Statistical analysis included 1) treatment (economic burden, side effects and time); 2) sleep quality; 3) whether influencing the care on family and the relationship with family members; 4) learning and work; 5) sports; 6) entertainment activities and interpersonal communication; 7) eating and dressing; 8)ability of doing housework and shopping; 9) frustration and embarrassment; 10) pain and itching. The above items could be further classified as: 1) treatment; 2) personal activities; 3) learning and work; 4) entertainment activities; 5) daily life; 6) personal feelings. The data judgment includes four grades, and the score from 0 to 3 represents none, mild, severe and very serious. The minimum score is 0 and the maximum is 30. The severity score is positively correlated with the impact on the quality of life in the patients with thyroid diseases.

Statistical analysis

All the data was analyzed using SPSS 17.0 statistical software. Measurement data was shown as mean±standard deviation ($\bar{x}$ ±s) and was analyzed using t-test. Enumeration data was analyzed using chi-square test and P<0.05 was considered statistical significance.

Results

Comparison of total operation time, thyroid resection time, intraoperative blood loss and postoperative drainage volume and complications between the observation group and control group

The total operation time, thyroid resection time, intraoperative blood loss and postoperative drainage volume in the observation group were significantly different from those in the control group (P<0.05) (Table 1).

Comparison of postoperative complications between the observation group and control group

There were 2 patients (2.04%) with deadlimb and transient hypocalcemia and 2 patients (2.04%) with hoarseness in the observation group, while there were 3 patients (3.06%) with deadlimb and transient hypocalcemia and 1 patient (1.02%) with hoarseness in the control group. The difference between the two groups had no significant difference (P>0.05)

Comparison of quality of life between the observation group and control group

The score of quality of life was significantly lower in the observation group (6.31±3.24) than in the control group (13.56±4.92) (P<0.05) (Table 2).

Table 1. Comparison of total operation time, thyroid resection time, intraoperative blood loss and postoperative drainage volume between the two groups

Operation	Group	Total operation time (min)	Thyroid resection time (min)	Intraoperative blood loss (g)	Postoperative drainage volume (mL)
Thyroidectomy+bilateral cervical lymph node dissection	Observation group	406.31±38.81	—	372.58±62.57	251.48±30.24
	Control group	552.57±56.21	—	571.31±75.29	351.31±41.42
Thyroidectomy+unilateral cervical lymph node dissection	Observation group	289.62±38.24	—	285.18±49.21	182.85±23.14
	Control group	392.42±45.19	—	431.57±54.68	294.56±32.82
Thyroid lateral lobe+ isthmus+contralateral lobe major resection	Observation group	86.32±7.21	33.71±5.03	24.62±4.51	32.74±10.32
	Control group	146.71±14.62	86.57±8.31	119.57±8.19	87.58±21.56
Thyroid lateral lobe+isthmus resection	Observation group	72.13±7.91	20.43±4.54	19.53±4.81	30.34±7.13
	Control group	126.67±12.63	65.46±5.78	98.43±10.15	85.28±18.57
Thyroidectomy	Observation group	108.42±8.48	42.36±6.24	28.42±5.26	39.43±10.43
	Control group	186.54±14.32	109.48±10.42	158.32±13.24	112.46±19.31

Table 2. Comparison of quality of life between the two groups

Group	n	Treatment	Personal activities	Learning and work	Entertainment activities	Daily life	Personal feelings	Summary
Observation group	98	0.31±0.17	0.56±0.45	0.83±0.52	0.84±0.52	1.24±3.13	2.83±1.72	6.31±3.24
Control group	98	2.32±1.52	2.92±1.41	0.96±1.31	1.82±0.51	2.84±2.51	2.79±1.74	13.56±4.92

Discussions

Surgery is the optimal treatment for thyroid diseases[8-10]. Conventional operation often left a large incision and the cervical scar may affect patients' quality of life, especially for the beauty and psychology of female patients. Endoscopic thyroidectomy is widely applied in clinical treatment of thyroid diseases[11-13], it can enlarge the surgical field, and will not hurt the recurrent laryngeal nerve, parathyroid glands or trachea. Surgeons should avoid put their scalpel towards the tissues above, and keep a distance not less than 3 mm. For some low risk thyroid cancer patients, lymph node dissection can be carried out at the same time to achieve an ideal outcome[14-16]. In our study, the total operation time, thyroid resection time, intraoperative blood loss and postoperative drainage volume were significantly less in the observation group than in the control group. The complications were observed in both of the groups, such as dead limb, transient hypocalcemia and hoarseness, but the difference was not significant between the two groups, indicating the efficacy of ultrasonic scalpel for open small incision thyroid operation traditional was better than that of conventional electrosurgical knife, which is consistent with the literatures[17-19].

Besides, when the open small incision thyroid operation is performed, the surgeon should be skilled and experienced to flexibly adjust the tension, and avoid hurting the surrounding tissues. Compared with conventional electrosurgical knife, ultrasonic scalpel has several advantages below: 1) Good homeostasis effect: making surgical field clear and avoiding hurting parathyroid glands and recurrent laryngeal nerve[6]; 2) Not affecting nerves and muscles: applying high-efficiency mechanical energy, avoiding the influence of electric current on normal electrophysiological activities, and suitable for patients with pacemaker or other metal materials[7]; 3) Less operation time: shortening the total operation time and thyroid resection time, reducing the incidence of postoperative in-

fections, and increasing the efficiency; 4) Reducing postoperative drainage volume; 5) Good incision recovery: small incision and quick recovery with less scar formation[20].

Taken together, ultrasonic scalpel application in open thyroid operation with small incision has advantages of short operation time, less blood loss and postoperative drainage volume, and better quality of life. It is worthy of further surgical application.

References

1. *Lombardi CP, Raffaelli M, Cicchetti A, et al. The use of "harmonic scalpel" versus "knot tying" for conventional "open" thyroidectomy: results of a prospective randomized study[J]. Langenbecks Arch Surg, 2009; 393(5): 627-631.*
2. *Hallgrimsson P, Loven L, Westerdahl J, et al. Use of the harmonic scalpel versus conventional haemostatic techniques in patients with Grave disease undergoing total thyroidectomy: a prospective randomised controlled trial[J]. Langenbecks Arch Surg, 2009; 393(5): 675-680.*
3. *Agcaoglu, S. Aliyev, J. Mitchell, et al. The Use of the Harmonic Scalpel Versus Knot Tying for Modified Radical Neck Dissection[J]. Surg. Innov. 2012; 19(4): 183-186*
4. *H. Markogiannakis, P. B. Kekis, N. Memos, L. Alevizos, et al. Thyroid surgery with the new harmonicscalpel: A prospective randomized study[J]. Surgery, 2011; 149(3): 411–415*
5. *B. Zarebczan, D. Mohanty, H. Chen, et al. A Comparison of the LigaSure and Harmonic Scalpel in Thyroid Surgery: A Single Institution Review[J]. Ann Surg Oncol., 2011; 18(1): 214-218*
6. *Samer S. Bessa, Alaa H. Abdel-Razek, Mohamed A. Sharaan, et al. Laparoscopic Cholecystectomy in Cirrhotics: A Prospective Randomized Study Comparing the Conventional Diathermy and the Harmonic Scalpel for Gallbladder Dissection[J]. Journal of Laparoendoscopic & Advanced Surgical Techniques, 2011; 21(1): 1-5.*
7. *J. A. Gracia, J. M. Ramirez, D. Callejo, et al. Efficiency and outcomes of harmonic device in transanal endoscopic microsurgery compared with monopolar scalpel [J]. Surgical Endoscopy, 2011; 25(10): 3209-3213*
8. *Miccoli P, Materazzi G, Miccoli M, et al. Evaluation of a new ultrasonic device in thyroid surgery: comparative randomized study[J]. Am J Surg. 2010; 199(4): 736-740*
9. *Yildirim O, Umit T, Ebru M, et al. Ultrasonic harmonic scalpel in total thyroidectomies[J]. Adv Ther. 2008; 25(5): 260-265.*
10. *Ecker T, Carvalho AL, Choe JH, et al. Hemostasis in thyroid surgery: harmonic scalpel versus other techniques- a meta-analysis[J]. Ototolaryngol Head Neck Surg., 2010; 143(11): 17-25.*
11. *Parker DJ, Krupa K, Esler R, et al. Use of the harmonic scalpel in thyroidectomy[J]. ANZ J Surg., 2009; 79(9): 476-480.*
12. *Jiang H, Shen H, Jiang D, et al. Evaluation the safety of the Harmonic Scalpel around the recurrent laryngeal nerve[J]. ANZ J Surg., 2010, 80(18): 822-826.*
13. *Koh YW, Park JH, Kim JW, et al. Clipless and sutureless endoscopic thyroidectomy using only the harmonic scalpel[J]. Surg Endosc, 2010; 24(13): 1117-1125.*
14. *Manouras A, Markogiannakis HE, Kekis PB, et al. Novel hemostatic devices in thyroid surgery: electrothermal bipolar vessel sealing system and harmonic scalpel[J]. Expert Rev Med Devices, 2008; 5(1): 447-466.*
15. *Mallur PS, Jethanamest D, Shemen LJ. Potential hazards of the harmonic scalpel[J]. Otolaryngol Head Neck Surg, 2009; 141(8): 303-304.*
16. *Ecker T, Carvalho AL, Choe JH, et al. Hemostasis in thyroid surgery: harmonic scalpel versus other techniques--a meta-analysis[J]. Otolaryngol Head Neck Surg, 2010; 143(25): 17-25.*
17. *Cooper DS, Doherty GM, Haugen BR, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer[J]. Thyroid, 2009; 19(12): 1167-1214.*
18. *Bandi G, Wen CC, Wilkinson EA, et al. Comparison of blade temperature dynamics after activation of Harmonic Ace scalpel and the Ultracision Harmonic Scalpel LCS-K5[J]. J Endourol, 2008; 22(9): 333-336.*
19. *Ortega J, Sala C, Flor B, et al. Efficacy and cost-effectiveness of the UltraCision harmonic scalpel in thyroid surgery: an analysis of 200 cases in a randomized trial[J]. J Laparoendosc Adv Surg Tech A, 2004; 14(11): 9-12.*
20. *Cordon C, Fajardo R, Ramirez J, et al. A randomized, prospective, parallel group study comparing the Harmonic Scalpel to electrocautery in thyroidectomy[J]. Surgery, 2005; 137(20): 337-341.*

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The effect of different fields on oxidant and antioxidant situations

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Abstract

The aim of this study has been to determine how the different fields have affected the oxidant and antioxidant parameters. 24 male participants not doing physical activity regularly whose average BMI was 21.79±1.79 (kg/cm^2), age was 21.70±1.54 (years), average height was 180.60±4.29 (cm) participated in the study. In this study, it was seen that the MDA, GSH and SOD pre-test parameters of both ATF and NTF groups were adversely affected from the physical activity. As a result, it was determined that the acute exercises caused to increase of MDA value and also decrease of SOD and GSH enzyme activities significantly in the post-exercise values of MDA, GSH and SOD parameters.

Key Words: Oxidative stres, natural turf field, artificial turf field.

Introduction

Turf fields have been seen as widely used areas not only for competitions but also for training. However, because of some reasons (climate, usage, maintenance) artificial turf fields (ATF) has been used as an alternative to the natural turf fields (NTF) in most of European countries. Besides it has been officially accepted by International Football Federation (FIFA) and European Football Federation (UEFA) that the third generation artificial turf fields have been thought as the most similar ones to the turf fields. It has been allowed to organize national leagues and official tournaments by UEFA and FIFA in most countries (UEFA, 2005; FIFA, 2005). The studies on the variables about both level of injury and physiological issues because of the activities on NTF and ATF have been considerably limited (Andersson et al 2008; Rocco et al 2009; Kerdok et al 2002). The effect of different kinds of fields on injury has been investigated in most of the studies (Rocco et al. 2009; Ekstrand et al 2006). Besides, some studies have purposed to determine the effect of physical activities on health (Sassi et al 2011; Andersson et al 2008; Kim et al 2012). However, it has not been seen any study to determine whether the different fields have affected the oxidant and antioxidant situations of athletes or not and also if they have, in what way they have affected. The human metabolism has produced many reactive oxygen metabolites and radicals in the course of its metabolic processes. These harmful molecules have caused to some diseases, DNA damage and aging effect (Lewandowski et al 2010; Antoncic-Svetina et al 2010; Elahi et al 2009; Essick and Sam 2010). The positive effects of physical activity on health have been widely known. In spite of these positive effects, it has been reported that the physical activity has affected the defense system in a negative way by increasing the free radical formation in some studies (Manna et al 2004; Atabek et al 2010; Urso 2003). It has been said that the acute exercises have increased the malondialdehyde (MDA) level which has showed the oxidative situation and have also decreased glutathione (GSH) and superoxide dismutase (SOD) parameters which has showed the antioxidant enzyme activities in both male and female participants (Codoner–Franch et al 2010; Miller-Kasprzak et al 2011). The aim of this study has been to determine how the different fields have affected the oxidant and antioxidant parameters.

Method

Participants

24 male participants not doing physical activity regularly whose average Body Mass Index (BMI)

was 21.79±1.79 (kg/cm^2), age was 21.70±1.54 (years), average height was 180.60±4.29 (cm) participated in the study. The participants were divided into Artificial Turf Field (ATF) and Natural Turf Field (NTF) groups randomly. In order to determine whether the participants could participate in the study or not, they were gone through fully physical examinations including EKG, routine biochemistry, hemogram and hormone tests and it was understood that none of them had any health problems to prevent physical activities. Some criteria were taken into consideration for participants to participate: (a) to play football in any football club regularly, (b) not to smoke and drink alcohol, (c) not to have any skeletal muscle disorder. The participants were informed about the study and each of them signed a voluntary participation form. Before the study, Ethics Committee approval (Protocol No: 2010/117) was taken from Inönü University Faculty of Medicine Regional Ethics Committee.

Table 1. The descriptive statistics of some physical and physiological parameters of participants

Parameters N (12)	ATF	NTF
	X±SD	X±SD
Age (years)	22.12±1.88	21.25±1.75
Height (cm)	180.37±7.30	178.62±4.86
Weight (kg)	72.25±6.79	67.37±6.43
Body Mass Index (kg/m^2)	22.37±2.55	21.00±1.69

Exercise Protocol

All the participants were performed 20 m shuttle run test designed by Leger et al. (1984; Le´ger, Mercier, Gadoury, & Lambert, 1988). The shuttle run test is mainly a physical activity that based on running through 20 m course; at the same time a sound signal is emitted from a prerecorded tape. Frequency of the sound signals is increased 0.5 km h^{-1} (0.1 m/s^{-1}) each minute from a starting speed of 8.5 km h^{-1} (2.4 m/s^{-1}) (one minute is equal in every stage) (Ruiz et al 2008). The participants were given instructions to turn back in accordance with the sound signals on the straight line. Test was ended when the participants couldn't run anymore or couldn't catch the sound signal twice in a row. The participants were motivated to perform better during the test. The exercise protocol was performed for both NTF and ATF groups in the same day and hour by taking into consideration the same criteria.

Collecting the Blood Sample

All the participants were wanted not to participate in any heavy exercises before 48 hours from the training day and not to have any food in the morning of training. 10 ml blood sample was drawn from the antecubital vein before 30 minutes and just after the exercise for pre-test evaluation. The vascular access was held open by probe in order not to pose a problem after the exercise. The blood samples were kept in -80 ^{o}C by the time the biochemical analyses were performed.

Data Analysis

The descriptive statistics of participants, Wilcoxon Signed Ranks test which was used to determine the significance level of outcomes between pre-test and post-test values of groups and Mann Whitney U test which was also used to determine the significance level between pre-test and post-test values of two groups were applied in the study. The level of significance was accepted as $p<0,05$, $p<0.01$ and the statistical results were reported as average ± standard deviation. Data were calculated by using SPSS 17.0 package program.

Results

The age, height, weight and BMI of the participants which was related to their personal characteristics were given in Table 1. MDA, a product of lipid peroxidation, has been accepted as an indirect marker to be used for oxidative stress. In this study, it was seen that the MDA, GSH and SOD pre-test parameters of both ATF and NTF groups were adversely affected from the physical activity. This situation could be explained as the increase in MDA value and the decrease in GSH and SOD values (Table .2, 3, $p<0.01$, $p<0.05$). The MDA pre-test values of ATF group increased from 11,71±3,43 nmol/ml to 13,26±2,84 nmol/ml. Besides

The MDA pre-test value of NTF group was seen as 13,03±3,69 nmol/ml before the exercise protocol and 16,04±3,65 nmol/ml after the exercise protocol (Table 2). These results showed that MDA values of both ATF and NTF groups increased just after the exercise. In the study, meanwhile the GSH pre-test value of ATF group was seen as 1,01±0,06 mg/dl, their post-test value was 1,01±0,06 mg/dl, on the other hand the pre-test value of NTF gro-

up significantly decreased from 1,12±0,02 mg/dl to 1,06±0,04 mg/dl (p<0.01). These findings showed that the GSH values of both ATF and NTF groups were lower after the exercise protocol and this increase was due to the exercise. In addition to these, it was determined that the SOD pre-test values significantly decreased from 3,16±0,29 mg/dl to 2,87±0,19 mg/dl (p<0.05).

When the MDA values, the pre-test and post-test oxidative stress marker of ATF and NTF groups, were compared, it was found that there was a statistically significant difference on behalf of ATF group (p=0.003). In other words, the MDA post-test values of ATF group (2,23±,1,21 nmol/ml) increased more than NTF group (1,50±0,51 nmol/ml). When the GSH parameters of ATF and NTF groups were compared, it was determined that NTF group (0,15±0,06 mg/dl) showed less change than ATF group (0,08±0,04 mg/dl). This situation was not statistically significant. It was also reported that there was no significant difference between the pre-test and post-test values of ATF and NTF groups (p=.248, table 3).

Discussion

Many different results were seen in the studies in order to find out how the MDA values changed depending on the intensity of exercise during physical activities. As some of them reported that the MDA values increased during physical activities (Miyazaki et al 2001; Ramel et al 2004; Cooper et al 2004), some of them found that the values decreased (Joo et al 2004; Dixon et al 2003) and also some of them determined that there had been no difference (Quindry et al 2003; Bloomer et al 2009; Munoz-Marin et al 2010). In this study, it was understood that there were statistically significant differences in the MDA values of ATF and NTF groups before the exercise and after the 20 m shuttle run. This study had the same findings with the studies to be applied for the effect of acute exercise on MDA value with different participants and different exercise protocols (Antoncic et al 2010; Grousarrd et al 2003). However, to determine that this difference occurred because of the field dissimilarity or intensity of exercise had importance so much in the sense of the originality and value of this study. At the end of the statistical analyses in order to understand the importance and contribution of field

Table 2. The MDA, GSH, SOD Pre-test and Post-test Values of Groups.

Group Parameters		N	ATF			NTF		
			X±SD	Z	p	X±SD	Z	p
MDA (nmol/ml)	Pre-test	12	11,71±3,43	-2,834	**0,000***	13,03±3,69	-2,675	**0,010***
	Post-test	12	13,26±2,84			16,04±3,65		
GSH (mg/dl)	Pre-test	12	1,11±0,08	-2,812	**0,000***	1,12±0,02	-2,533	**0,011***
	Post-test	12	1,01±0,06			1,06±0,04		
SOD (mg/dl)	Pre-test	12	3,19±0,25	-2,521	**0,012***	3,16±0,29	-2,521	**0,012***
	Post-test	12	2,74±0,19			2,87±0,19		

(ATF: Artificial Turf Field, NTF: Natural Turf Field, MDA: Malondealddehyde, GSH: Glutathyon, SOD: Superoxide Dismutase, p: Significant differ)

Table 3. The Mann Whitney U Results of the MDA, GSH, SOD Pre-test and Post-test Values of ATF and NTF Groups

Parameters	Group	N	The Differences of Pre-test and Post-test X±SD	Mann Whitney U	p
MDA (nmol/ml)	ATF	12	2,23±,1,21	4,000	**0.003****
	NTF	12	1,50±0,51		
GSH (mg/dl)	ATF	12	0,08±0,04	15,500	0.080
	NTF	12	0,15±0,06		
SOD (mg/dl)	ATF	12	0,40±0,23	21,000	0.248
	NTF	12	0,50±0,21		

(ATF: Artificial Turf Field, NTF: Natural Turf Field, MDA: Malondealddehyde, GSH: Glutathyon, SOD: Superoxide Dismutase, p: Significant differ)

dissimilarity, it was seen that ATF participants had higher MDA difference than NTF participants after the acute exercise and this difference was statistically significant ($p<0.05$). Besides in the study, the acute exercise applied on ATF and NTF participants had affected and also decreased the GSH value and it was seen that this difference was statistically significant. In the studies by Bersoza et al (2011) and Revan et al (2010), it was found that the acute exercise decreased the GSH enzyme activity similarly with this study; on the other hand in the study by Deminice et al (2010), it was reported that the acute exercise increased the GSH enzyme activity statistically. When the GSH enzyme activity differences of ATF and NTF participants in terms of field dissimilarity were compared, it was determined that there was no significant difference ($p=0.080$). It was also seen that there was statistically significant decrease between the pre-test and post-test values of both ATF and NTF participants in terms of SOD enzyme activity. As this result had the same results with the studies by Aguilo et al (2005) and Shin et al (2008), on the other hand it did not have the same results with the studies by Bersoza et al (2011) and Pinho et al (2010). It was understood that the field dissimilarity didn't lead to statistical significance in the sense of SOD enzyme activity.

As a result, it was determined that the acute exercises caused to increase of MDA value and also decrease of SOD and GSH enzyme activities significantly in the post-exercise values of MDA, GSH and SOD parameters. In spite of the fact that these findings didn't show similarity with the some studies, they were similar with the most of studies in the literature. In addition to these, it was reported that MDA parameters affected much more from the acute exercises on ATF in terms of field dissimilarity and this situation was statistically significant. In other words, it can be said that the risk of oxidative stress because of the acute exercises on ATF is higher in the view of such information. Yet, this subject is still needed to be searched by other researchers with the larger sample group.

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References

1. *Andersson H, Ekblom B & Krustrup P. Elite football on artificial turf versus natural grass: Movement patterns, technical standards, and player impressions, Journal of Sports Sciences, January 15th 2008; 26(2): 113 – 122.*
2. *Antoncic-Svetina M, Sentija D, Cipak A, Milicic D, Meinitzer A, Tatzber F, Andrisic L, Zelzer S, Zarkovic N. (2010) Ergometry induces systemic oxidative stress in healthy human subjects. Tohoku J Exp Med. 221(1): 43-8*
3. *Atabek HÇ, Demir S, Pinarbaşili RD, Gündüz N. "Effects of different resistance training intensity on indices of oxidative stress". Journal of Strength and Conditioning Research. (2010), 24(9): 2491-7.*
4. *Bloomer JR, Cole JB. (2009). Relationship between blood lactate and oxidative stress biomarkers following acute exercise. Open Sports Medicine Journal. 3: 44-48.*
5. *Cooper CE, Vollaard NBJ, Choueiri T, Wilson MT. (2002). Exercise, free radicals and oxidative stress. Biochem Soc Trans, 30(2): 280-5.*
6. *Di Michele R, Di Renzo AM, Ammazzalorso S, and Merni F. (2009). Comparison of physiological responses to an incremental running test on treadmill, natural grass, and synthetic turf in young soccer players. Journal of Strength and Conditioning Research. 23(3): 939-945.*
7. *Dixon CB, Robertson RJ, Goss FL, Timmer JM, Nagle E, Evans RW. (2003). Effect of resistance training status on free radical production and muscle damage following acute exercise. Med Sci Sports Exerc. 35(5): 157-162.*
8. *Ekstrand J, Timpka T, Hagglund M, Risk of injury in elite football played on artificial turf versus natural grass: a prospective two-cohort study, Br J Sports Med 2006; 40: 975–980.*
9. *Elahi MM, Kong YX, Matata BM. (2009) Oxidative stress as a mediator of cardiovascular disease. Oxid Med Cell Longev. 2(5): 259-69*
10. *Essick EE, Sam F (2010) Oxidative stress and autophagy in cardiac disease neurological disorders, aging and cancer. Oxid Med Cell Longev. 3(3): 167-77*
11. *FIFA (2005). FIFA quality concept for artificial turf guide (pp. 1 – 43). Zurich: FIFA.*
12. *Groussard C, Rannou-Bekono F, Machefer G, Chevanne M, Vincent S, Sergent O. et all. (2003). Changes in blood lipid peroxidation markers and antioxidants after a single sprint anaerobic exercise. Eur J Appl Physiol, 89: 14-20.*

13. *Hartmann A, Niess AM. (1998). Oxidative DNA damage in exercise. Pathophysiology 5: 112.*

14. *Joo M, Maehata E, Tetsuo A, Akiko I, Murai F, Noboru M. (2004). The relationship between exercise-induced oxidative stress and the menstrual cycle. Eur J Appl Physiol, 93(1-2): 82-6.*

15. *Kerdok, AE, Biewener, AA, Mcmahon, TA, Weyand, PG, and Herr, HM. Energetics and mechanics of human running on surfaces of different stiffnesses. J Appl Physiol. 92: 469–478, 2002.*

16. *Kim S, Yang SJ, Kim HH, Yeo IY, Shin DC, Lim YW. Health Risk Assessment of Lead Ingestion Exposure by Particle Sizes in Crumb Rubber on Artificial Turf Considering Bioavailability, The Korean Society of Environmental Health and Toxicology*

17. *Le´ ger, LA, Lambert A, Goulet A, Rowan C, & Dinelle Y. (1984). Capacity aerobic des Quebecois de 6 a 17 ans – test Navette de 20 meters avec paliers de 1 minute. Canadian Journal of Applied Sport Sciences, 9, 64–69.*

18. *Le´ ger, LA, Mercier D, Gadoury C, & Lambert J. (1988). The multistage 20 meter shuttle run test for aerobic fitness. Journal of Sports Sciences, 6, 93–101.*

19. *Lewandowski P, Hübner-Woźniak E, Effects of competitive pentathlon training on the antioxidant defense components, Biomedical Human Kinetics, 2, 78 – 80, 2010*

20. *Manna, I, Jana, K, and Samanta, PK. Intensive swimming exercise–induced oxidative stress and reproductive dysfunction in male wistar rats: Protective role of alpha-tocopherol succinate. Can J Appl Physiol 29: 172–185, 2004.*

21. *Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Ha S, Haga S, Ji LL. (2001). Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. Eur J Appl Physiol, 84: 1-6.*

22. *Munoz-Marin D, Olcina G, Timon MC, Caballero MJ, Maynar M. (2010). Effect of different exercise intensities on oxidative stress markers and antioxidant response in trained cyclists. J Sports Med Phys Fitness. 50(1): 93-8.*

23. *Packer, L. Oxidants, antioxidant nutrient and the athletes. J Sports Sci 15: 353–263, 1997.*

24. *Quindry JC, Stone WL, King J, Broeder CE. (2003). The effects of acute exercise on neutrophils and plasma oxidative stress, Med Sci Sports Exerc. 35(7): 1139-45.*

25. *Ramel A, Wagner KH, Elmadfa I. (2004). Plasma antioxidants and lipid oxidation after submaximal resistance exercise in men. Eur J Nutr, 43: 2-6.*

26. *Ruiz JR, Silva G, Oliveira N, Ribeiro JC, Oliveira JF & Mota J. Criterion-related validity of the 20-m shuttle run test in youths aged 13–19 years, Journal of Sports Sciences, 2009; 27(9): 899–906.*

27. *Sassi A, Stefanescu A, Menaspa' P, Bosio A, Riggio M, and Rampinini E. The cost of running on natural grass and artificial turf surfaces, Journal of Strength and Conditioning Research, 25(3): 606–611.*

28. *UEFA (2005). FIFA quality concept: Handbook of test methods and requirements for artificial turf football surfaces. Nyon, Switzerland: UEFA.*

29. *Urso ML, Clarkson PM. Oxidative stress, exercise and antioxidant supplementation. Toxicology. 2003; 189: 41-54*

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Effects of total panax notoginseng saponins plus flutamide on 25 cases of advanced prostate cancer patients

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Abstract

Objective: To observe the clinical effects of total panax notoginseng saponins (tPNS) plus flutamide on prostate cancer (PCa) patients.

Methods: 55 cases of PCa patients were randomly divided into a trial group (30 patients) and a control group (25 patients). The control group were administered with flutamide (245 mg, tid), on the basis of which the trial group were administered with tPNS (1.5 mL, qd) intravenously. After they were treated for 2 months, the prostate volumes, the dysuria statuses, the levels of serum f/tPSA and the treatment efficacies were compared.

Results: All the target testing items of the two groups did not differ significantly before the treatment. In contrast, their prostates shrank apparently, dysuria was alleviated evidently, but the levels of serum f/tPSA increased significantly after the treatment. Meanwhile, the statuses of the trial group were even better. The overall effective rate of the trial group (90.0%) was significantly higher than that of the control group (72.72%)($P<0.05$).

Conclusion: The PCa patients were treated better after being administered with tPNS plus flutamide compared to flutamide alone, which allows further investigation and promotion.

Key words: Prostate cancer, flutamide, total panax notoginseng saponins, clinical treatment efficacy.

Introduction

Prostate cancer (PCa), which is one of the common malignant tumors, occurs in various regions and races differently. The incidence rate and and the morbidity rate of PCa rank 1st and 2nd in all male cancers (2010, USA) [1]. More younger people in China are prone to PCa in recent years. Currently, the incidence of PCa ranks third among the male urogenital system malignant tumors [2]. The symptoms of PCa do not manifest early, and 39% of PCa after surgeries and radiotherapy may recur or metastasize [3]. Therefore, auxiliary drug treatment after chemotherapy and radiotherapy will improve the quality of life and prolong the survival time.

Total panax notoginseng saponins (tPNS) is the pharmaceutical component extracted from the root of a traditional Chinese medicine panax notoginseng. The injection of tPNS (also known as Xuesaitong injection) is a drug that has been widely used to treat cardiovascular diseases such as coronary heart disease and cerebral thrombosis, etc. [4]. It has been recently reported that tPNS could inhibit the proliferation of human liver cancer cells, as well as resist the oxidation and proliferation of human colon cancer cells that lead to apoptosis [5]. However, the effects of tPNS on prostate cancer are still not clear. Thereby motivated, the study aims to provide experimental evidence for the auxiliary treatment of PCa by tPNS and the promotion of tPNS clinical use by observing the effects of tPNS plus flutamide on PCa.

Materials and methods

General information

55 cases of advanced stage PCa patients (Age: 45-76, average age: 66±2.5) recruited in our hospital from May 2011 to May 2012 were selected. All patients were untreated with dysuria, bladder irritation symptoms such as pollakiuria and urgent urination, etc., visible hematuria and hieralgia. The patients were diagnosed by CT, color Doppler ultrasonic, bone scan, radioimmune PSA check and prostate needle biopsy. The patients were voluntarily divided into the control group (25 cases, 45-74 years old, average: 67±1.3 years old) and

the trial group (30 cases, 46-77 years old, average: 66±2.4 years old). The age and the gender of the two groups did not differ significantly.

Treatment method

The control group were administered with flutamide (245 mg, tid), on the basis of which the trial group were administered with tPNS (1.5 mL, qd; Xuesaitong injection, patch No.: 20100513, Kunming Xingzhong Pharmaceutical Co., Ltd.) intravenously. Both groups were treated for 2 months [6].

Treatment efficacy criteria [7]

The alleviated symptoms allow the treatment to be effective include: 1) a >50% reduction of prostate volume or disappeared nodule lesions confirmed by anal examination and type-B ultrasonic; 2) significantly mitigated dysuria; 3) mitigated pain for more than 2 months; 4) a >50% decline of blood PSA; 5) a >50% reduction of X-ray metastatic bone lesion area; 6) apparently improved systemic situation. Besides, the treatment leading to no less than 2 above indexes was considered markedly effective.

Observation index

Dysuria (pollakiuria, nocturia, urine bifurcation, dripping urine), serum PSA levels before and 5 weeks after medication, (BAYER kit), prostate volume and texture confirmed by anal examination, metastatic bone lesion area observed by X-ray, blood routine examination, liver function and kidney function.

Statistical analysis

SPSS 15 was used to perform statistical analysis. The measurement data were expressed in mean ± standard deviation. The comparison per se and the comparison between groups were performed by paired t test and grouped t test, respectively. The numeration data were compared by the Chi square test. P<0.05 was considered statistically significant.

Results

Comparison of prostate volumes before and after treatment

The prostate volumes of the two groups before treatment did not differ significantly, but their prostates shank by 31.5% and 56.71%, respectively. The shrinkage rate of the trial group was significantly higher than that of the control group (P<0.01) (Table 1).

Comparison of dysuria situation before and after treatment

The typical symptoms of PCa include dysuria, pollakiuria and etc. The dysuria of the control group (96.0%) and the trial group (93.33%) before treatment did not differ significantly, which decreased to 31.5% and 56.71%, respectively. The dysuria incidence of the trial group was significantly lower than that of the control group (P<0.01) (Table 2).

Table 1. Comparison of prostate volumes before and after treatment

Group	Case number	Volume before treatment (mL)	Volume after treatment (mL)	Shrinkage rate (%)
Control	25	64.9±2.8	44.7±2.4	31.5
Trial	30	65.8±2.7	29.0±2.3	55.92*

**: compared to the control group, P<0.01*

Table 2. Comparison of dysuria situation before and after treatment

Group	Case number	Number before treatment	Number after treatment
Control	25	24	22
Trial	30	28	4

Table 3. Comparison of serum f/tPSA ratios before and after treatment

Group	Case number	Number before treatment	Number after treatment
Control	25	0.12±0.09	0.21±0.04
Trial	30	0.13±0.04	0.25±0.08*

**: compared to the control group, P<0.05*

Table 4. Comparison of the treatment efficacy of the two groups

Group	Case number	Markedly effective	Effective	Effective rate
Control	25	6	13	76.0%
Trial	30	14	13	90.0%*

**: compared to the control group, P<0.05*

Comparison of serum f/tPSA ratios before and after treatment

PSA (prostate specific antigen) is the most sensitive marker of PCa that is excreted by prostate acinus and ductal epithelial cells. The early diagnosis rate of PCa can be substantially increased by measuring the ratio of serum free PSA (fPSA) level to total PSA (tPSA) level. The f/tPSA ratios of the two groups before treatment did not differ significantly, but the values rose drastically after treatment. The f/tPSA ratio of the trial group was significantly higher than that of the control group (P<0.05) (Table 3).

Comparison of the treatment efficacy of the two groups

The overall effective rate of the trial group (90.0%, markedly effective: 14 cases, effective: 13 cases) was significantly higher than that of the control group (76.0%, markedly effective: 6 cases, effective: 13 cases) (P<0.05) (Table 4).

Discussion

More younger people are subject to PCa. Agatstein et al. [8] reported that PCa focus was found in 18% of the prostate hyperplasia patients by needle biopsy. Coplen et al. [9] also confirmed that cancer cells were simultaneously found in 14% of the prostate hyperplasia patients guided by transrectal ultrasound.

Endocrine therapy is primarily used to treat the advanced stage PCa. However, the original PCa almost develops to androgen independent PCa and further hormone refractory PCa after the median time of 12-32 months [7]. The median survival time of the hormone independent PCa is only 10-22 months [10].

Antiandrogenic drugs, such as flutamide, are currently being widely applied as endocrine drugs and given priority in the treatment of advanced stage PCa. Although flutamide outweighs other anti-androgen drugs in the specific hormone activity, the maintaining of libido and sexual function as well as the individual clinical use, it may easily lead to drug resistance in case of being used alone. As a mixture of various saponins [11], tPNS and the monomers therein have been reported to resist malignant tumors such as liver cancer, cervical cancer and colorectal cancer, etc. [12]. In addition, tPNS is able to inhibit the proliferation of PCaPC-3 cells, significantly inhibit the migration of PC-3 cells, and reduce the expressions of PCNA, VCAM-1 and MMP-2 in PC-3 cells [13]. The results herein show that the combination of tPNS and flutamide significantly lowered the prostate volumes, alleviated dysuria situation and elevated the serum f/tPSA levels, revealing that the treatment efficacy and the overall effective rate significantly outweighed those of flutamide alone.

In short, the remarkably better treatment efficacy of tPNS plus flutamide verified in this study is worthy of further research and clinical application.

References

1. *D'Amico AV, Smith MR. Screening for Prostate Cancer. N Engl J Med. 2012; 16;367(7): e11.*

2. *Wang X, Guo Z, Wang HT, Si TG. [Clinical features and prognostic analysis in prostate cancer patients under 59 years of age: a report of 72 cases]. Zhonghua Yi Xue Za Zhi. 2012; 22, 92(19): 1300-3.*

3. *Wever EM, Draisma G, Heijnsdijk EA, de Koning HJ. How does early detection by screening affect disease progression? Modeling estimated benefits in prostate cancer screening. Med Decis Making. 2011; 31(4): 550-8.*

4. *Liu H, Yang J, Du F, et al. Absorption and disposition of ginsenosides after oral administration of Panax notoginseng extract to rats. Drug Metab Dispos. 2009; 37(12): 2290-8.*

5. *Zhou D, Hong Z, Wang YJ, Chi ZF, Luo BY, Di Q, Chen KM. [Clinical study on effect of sanqi tongshu capsule in treating ischemic stroke: multicenter clinical trial]. Zhongguo Zhong Yao Za Zhi. 2008; 33(22): 2692-6.*

6. *Li K, Yuan YF. Therapeutic Effects of tPNS plus Flutamide on Prostate Cancer. Anti-tumor Pharmacy. 2012; 4(2): 148-149.*

7. *Lu H, Hu XJ, Wanf SQ. Efficacy of Endocrine Therapy Combined with Radiotherapy for Locally Advanced Prostate Cancer. Chinese General Practice. 2012; 7(15): 2411-2412.*

8. *Agatstein EH, Hernandez FJ, et al. Use of fine needle aspiration for detection of stage A prostatic carcinoma before transurethral resection of the prostate: a clinical trial. J Urol. 1987; 38(3): 551-3.*

9. *Coplen DE, Austin PF, Yan Y, Dicke JM. Correlation of prenatal and postnatal ultrasound findings with the incidence of vesicoureteral reflux in children with fetal renal pelvic dilatation. J Urol. 2008; 180(4): 1631-4.*

10. *Li X, Ma B. Current Status of Endocrine Therapy of Prostate Cancer. Medical Recapitulate. 2011; 17(21): 3266-3277.*

11. *Guo YY, Mai WH, Liu HW, Guo DR. [Clinical study of intermittent therapy with flutamide and enanton on advanced prostate cancer after castration]. Chinese Journal of Clinical Research. 2011; 8(24): 659-661.*

12. *Huang WQ. [Clinical effect and pharmacological analysis of panax notoginseng]. Med Inform. 2011; 1(365): 304-305.*

13. *Su M ,Wen JK. [Total panax notoginseng saponins inhibit the migration of vascular smooth muscle cells]. Chinese Pharmacological Bulletin. 2010; 26(10): 1326-9.*

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Relation between cyclooxygenase-2 expression and neoangiogenesis in gastric cancer

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Abstract

Objective: Neoangiogenesis is a process of forming new tumor blood vessels. It is crucial for survival, further development and metastasis of tumors. Some studies suggest the possible correlation between the activity of cyclooxygenase 2 (COX-2) and neoangiogenesis. This study investigated whether is there a correlation between COX-2 activity in the gastric cancer tissue and the neoangiogenesis.

Material and methods: We analyzed 60 gastric cancer samples, fixed in formalin, paraffin embedded and 20 samples of gastric mucosa with chronic inflammation taken from the same patients that served as control group. In order to visualize COX-2 and the old and newly formed blood vessels (respectively) immunohistochemical staining with COX-2, CD34 and CD105 antibodies have been used.

Results: In the group of gastric cancer samples significantly higher levels of COX-2 were found compared to the group of gastric mucosa samples with gastritis. COX-2 demonstrated a statistically significant correlation with neoangiogenesis visualized with the protein CD105, but it had no effect on microvessel density (MVD) visualized with the CD34 antibody, or the size of primary tumor and invasion of the lymph glands. The number of CD105 positive newly formed blood vessels was significantly higher in the study group, but did not affect the size of primary tumor, or invasion of the lymph glands.

Conclusion: The study confirmed the existence of correlations between protein expression of COX-2 in gastric cancer samples and the number of CD105 positive newly formed blood vessels.

Key words: Gastric cancer, neoangiogenesis, immunohistochemistry, COX-2, CD105, CD34.

Introduction

In 1972 Judah Folkman set a milestone for investigating solid tumors in humans, when he proposed hypothesis that the growth of solid tumors is angiogenesis dependent (1). Tumor angiogenesis, a process also known under the term of neoangiogenesis, is a process of forming new intra-tumor blood vessels, which is crucial for survival, the further development and metastasis of tumors. This investigation opened a whole new road for tumor studies that was mostly directed to possible inductors and inhibitors of tumor angiogenesis. Cyclooxygenase 2 (COX-2), inducible enzyme is included in cyclooxygenase pathways, it is absent in most tissues under normal "resting" conditions and stimulates production of the prostaglandins (PGs) that are involved in inflammatory conditions (2). COX-2-mediated PG biosynthesis has been suggested to be involved in the development of cancer based on elevated levels of PGs, especially prostaglandin E2 (PGE2), in cancer tissues (3). COX-2-derived PGE2 can contribute to tumor development through several mechanisms including promotion of angiogenesis, inhibition of apoptosis, increased invasiveness/motility, and modulation of inflammation and immune responses (4). PGE2 then induces entry of hypoxia inducible factor-1α (HIF-1α) from the cytosol into the nucleus, inducing transcription of vascular endothelial growth factor (VEGF) and in this way, COX-2 is able to induce the transcription and the expression of VEGF protein (5). Eberhart et al. conducted a study that investigated COX-2 gene expression via Northern blot analysis of poly(A)+ RNA isolated from human colorectal cancers, adenomas, and accompanying normal mucosa and concluded that COX-2 expression seems to be increased in a subset of adenomas and COX-2 may provide an attractive therapeutic target in colorectal neoplasm (6,7). Widely accepted measure of tumor neoangiogenesis is microvascular density, especially after Weidner et al. in the early 1990s, showed that measurement of microvessel density within isolated regions of high vessel concentration (i.e., hotspots) was a prognostic indicator

for human breast and prostate carcinomas (8-10). Following these reports many types of cancers have been investigated regarding influence of COX-2 to their angiogenesis, including breast cancer, prostate cancer and pancreatic cancer. Our aim was to examine whether there is a correlation between COX-2 activity in the tissue and the neoangiogenesis.

Material and methods

Patient selection

This study included tissue specimen obtained from 60 patients, following radical gastrectomy and regional lymphadenectomy, diagnosed with gastric cancer at the Department of Pathology University of Sarajevo School of Medicine. Twenty samples of gastric mucosa with gastritis taken from the same patients served as control group. The samples were fixed in formalin, paraffin embedded and cut into 3-5 μm sections.

The study group consisted of 42 men and 18 women with average age of 63,6 years at the time of diagnosis.

Following surgical resection, we analyzed classical pathohistological parameters in accordance to the American Joint Committee on Cancer recommendations (AJCC) including TNM (tumor, node, metastases) stage determined by the depth of invasion, the involvement of the lymph nodes and distant metastasis (11). When determining histological type of the tumor we used the WHO classification for gastric cancer (12).

Immunohistochemical analysis

In order to investigate the eventual overexpression of COX-2 in the gastric cancer samples and its relation to the neoangiogenesis we used immunohistochemical staining. Both investigated and control group samples were subject to staining with COX-2, CD105 and CD34 antibodies. Antibodies CD105 and CD34 were used to visualize blood vessels, relatively according to widely accepted practice that CD105 stains newly formed blood vessels, as it is considered to currently golden standard for visualization of neoangiogenesis, and CD34 was used to visualize previously existing blood vessels (13-16).

The routine staining processes was performed. All the issue specimens were fixed in 10% neutral formalin and embedded in paraffin. Briefly, 5-μm sections of tumor tissues and non-neoplastic (peritumoral) gastric mucosa were mounted on poly-D-lysine coated slides. Thin sections were deparaffinized in xylene and rehydrated in a series of ethanol solutions (100%, 90%, and 80%) for 5 minutes each, washed in distilled water and three times in 0.05 mol/L PBS (pH 7.4), immersed in 10 mmol/L citrate buffer (pH 6.0) and put in a microwave for 5 min at 60°C for antigen retrieval. Then they were placed in methanol containing 3% H_2O_2 for 30 min at 4°C to block endogenous peroxidase activity and incubated with rabbit serum for 10 min to block non-specific antibody binding sites. After blocking the endogenous peroxidase and non-specific binding, the sections were incubated with primary antibodies, anti-COX-2 mouse monoclonal antibody (dilution range 1:80, DAKO, Denmark), anti-endoglin (CD105) mouse monoclonal antibody (Novocastra clone 4G11, IG class G2a) and anti-human CD34 mouse monoclonal antibody (DAKO clone BI-3C5, class I). The primary antibodies were applied at a working concentration and incubated for 2 hours at 4°C. The secondary antibody and the avidin-biotin-peroxidase complex (ABC) were applied to slides. 3,3 ’- Diaminobenzidine (DAB) was used as a chromogen and sections were counterstained with Mayer’s hematoxylin. Negative controls were obtained by replacing the primary antibody by non-immunized rabbit or mouse serum.

Quantification of Immunostaining

The COX-2 immunohistochemical expression was determined by immunohistochemical score (IHS). This was calculated by combining the percentage of positive stained cells with staining intensity score (8). The percentage of positive cells was categorized as 0 = negative; 1=1-10% positive cells; 2=11-50% positive cells; 3=51-80% positive cells; 4=≥81% positive cells. The staining intensity was categorized as 0=negative; 1= weak; 2 =moderate; 3 =strong. Raw data were converted to HIS by multiplying the quantity score (o-4) by the staining intensity score (0-3). Theoretically, the IHS can range from 0 to 12. An IHS of 9-12 was considered a strong immunoreactivity; 5-8 =moderate; 1-4=weak; and 0=negative. In statistical analysis, COX-2 scores were placed in positive (1-12) and negative (0) group.

Quantification of tumor microvasculature in this investigation was evaluated based on number of fully formed blood vessels with lumen, by counting number of blood vessels in ten HPF (x40).

Statistical Analysis

Statistical analyses were performed with SPSS 19.0 software (SPSS Inc, Chicago, USA). The correlation among the expression of COX-2, CD105 and CD34 with clinicopathological parameters (gender, age, size, tumor differentiation, lymphovascular invasion, number of lymph nodes involved) were calculated by Student's *t*-test, chi-square correlation test and Spearman's coefficient of correlation as appropriate. The statistical significance level was defined as $p<0.05$.

Results

The observed pattern of COX-2 protein expression in the study among gastric tumor samples and mucosa with gastritis group is displayed in Figure 1. In 38 (63,3%) samples, immunohistochemical expression of COX-2 was weakly expressed and in 1 (1,7%) case there was a moderate expression while 21(35%) samples of gastric cancer showed no expression of COX-2 protein. Eight cases (40%), in gastritis group expressed weak immunoreactivity of COX-2 protein and six cases (30%) expressed negative, relatively moderate immunoreactivity. COX-2 protein immunoreactivity demonstrated statistically significant difference between gastric cancer samples and gastritis samples group (p<0,05) (Figure 1), with statistically significant difference among gastric cancer group samples regarding different grades of differentiation (p<0,05) with highest expression of positively stained samples in tumor grade II (Table 1.).

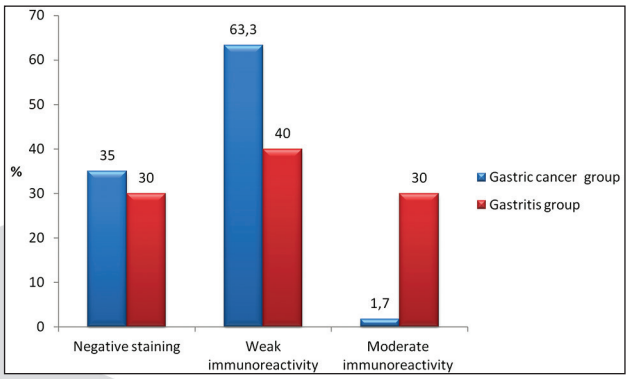

Figure 1. Expression of COX-2 protein in 60 specimen of gastric cancer and 20 samples of gastric mucosa with gastritis

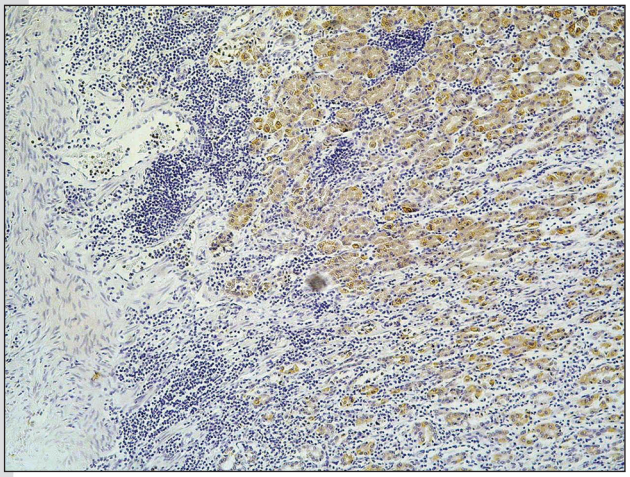

Figure 2. Immunohistochemical staining of gastric cancer mucosa samples with COX-2 (x20)

Average number of newly formed CD105 positive blood vessels was 9,58 when counted in 10 HPF with minimal number of 0 and maximal of 36. The samples in control group showed no CD105 positive blood vessels, what demonstrate

Table 1. Correlation of COX-2 protein expression and in regard to the tumor grade in 60 specimen of gastric cancer

COX-2 expression		Degree of tumor differentiation				Total
		I	II	III	IV	
Negative immunoreactivity	Number of samples	10	0	4	7	21
	%	66,7	,0	26,7	46,7	35,0
Weak immunoreactivity	Number of samples	5	15	11	7	38
	%	33,3	100,0	73,3	46,7	63,3
Moderate immunoreactivity	Number of samples	0	0	0	1	1
	%	,0	,0	,0	6,7	1,7
Total	Number of samples	15	15	15	15	60
	%	25,0	25,0	25,0	25,0	100,0

statistically significant difference between gastric cancer samples and gastritis samples groups obtained by variance analyze (F=18,429; p<0,05) (Table 2). The number of CD105 positive blood vessels showed no significant difference regarding single grades (F=0,862; p>0,05) in gastric cancer group nor to the connection to the tumor size (F=0,593; p>0,05) or regional lymph node involvement (F=0,157; p>0,05).

Average number of CD34 positive blood vessels was 28,4 for gastric cancer samples group while it was 27,75 in gastritis samples group. There was no statistically significant difference between gastric cancer group and gastritis samples group regarding number of CD34 positive blood vessels (F=0,057; p>0,05) and it did not affect size of the primary tumor (F=0,203; p>0,05) nor the regional lymph nodes involvement (F=1,221; p>0,05).

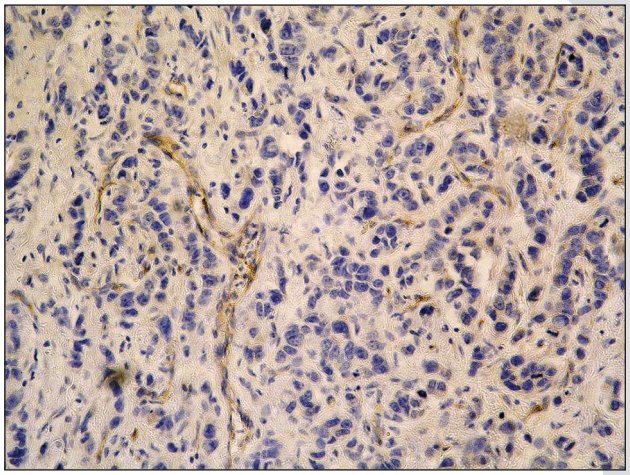

Figure 3. Immunohistochemical staining of newly formed blood vessels in gastric cancer mucosa samples with CD105 (x20)

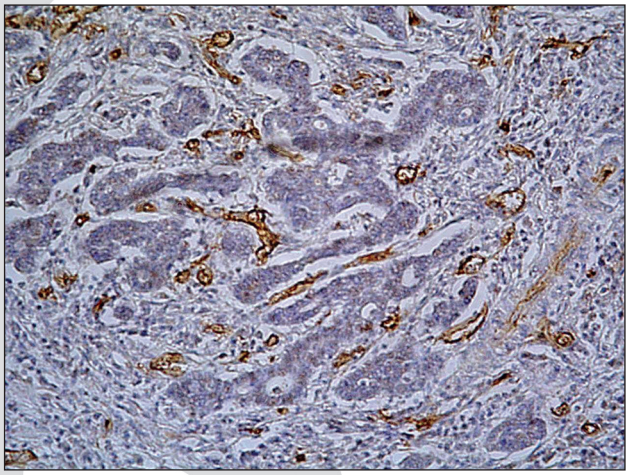

Figure 4. Immunohistochemical staining of previously formed blood vessels in gastric cancer mucosa samples with CD34 (x20)

Table 2. Number of CD 105 positive blood vessels in 60 specimen of gastric cancer and 20 samples of gastric mucosa with gastritis

CD105						
	Number of samples	**AM⁺**	**Standard Deviation**	**Standard error**	**Minimal value**	**Maximal Value**
Gastric cancer samples	60	9,5833	9,94100	1,28338	,00	36,00
Gastritis samples	20	,0000	,00000	,00000	,00	,00
Total	80	7,1875	9,55211	1,06796	,00	36,00

⁺ *AM-arithmetic mean*

Table 3. Correlation between COX-2 protein expression and number of newly formed (CD 105 positive) and previously formed (CD34 positive) blood vessels

		COX-2	**CD105**	**CD34**
Grade	Pearson correlation	,039	,113	-,021
	p- value	,768	,391	,871
	No.	60	60	60
COX-2	Pearson correlation		**-,347***	-,056
	p- value		**,007**	,672
	No.		60	60
CD105	Pearson correlation			,131
	p- value			,320
	No.			60

**Significance of correlation on level of 0,01.*

Analyzing the connection between COX-2 protein expression regarding formation of new blood vessels stained with CD105 Pearson test demonstrated that there was a statistically positive correlation (Ro= -0,347; p=0,007), what points out that elevated values of COX-2 protein promotes neoangiogenesis (Table 3). The same test when analyzing link o of COX-2 protein expression to the number of CD34 positive blood vessels showed that there is a correlation but not on statistically significant level (Ro= -0,056; p=0,672) (Table 3).

Discussion

In our study COX-2 protein immunoreactivity demonstrated statistically significant difference between gastric cancer samples and gastritis samples group (p<0,05) (Figure 1), with statistically significant difference among investigated group samples regarding different grades of differentiation (p<0,05) with highest expression of positively stained samples in tumor grade II.

Our finding of COX-2 expression in the gastric cancer samples group matches those of Mao et al. who believe that overexpression of COX-2 can play an important role in gastric cancer carcinogenesis. Mao conducted large sample study with 104 gastric cancer samples and 79 normal gastric mucosa specimen using tissue microassay and found statistically significant difference in COX-2 expression between investigated samples in both groups (17). Yu et al. investigated 30 samples of gastric cancer and normal mucosa and concluded that there is a statistically significant difference in COX-2 expression among groups with significance related to the pathological stage and regional lymph nodes involvement (18). Uefeji et al. published study based on samples of 42 patients that investigated expression of COX-2, prostaglandin levels and microvascular density using Immunoblot analysis. Out of total number of included patients, 34 (74%) of them demonstrated overexpression of COX-2 with increased production of PGE2 and angiogenesis what demonstrate possible link to development of gastric cancer (5). Sun et al. concluded following their study that investigated 96 patients with gastric cancer and 30 samples of cancer free mucosa from same patients that there are significantly higher values of COX-2 expression in the investigated group and those were associated with stages III and IV, relatively with lymph nodes metastasis (19).

One of the studies that extensively investigated visualization of neoangiogenesis was that performed by Ding et al. who investigated expression of CD34 and CD105 in benign and malignant lesions of stomach. They concluded that CD34 was universally expressed while CD105 expression was limited to malignant tissue specimen (20).

In our study there was link between the COX-2 overexpression and the number of CD105 positive blood vessels with a statistically significant positive correlation (Ro=0,303; p<0,05), directly supporting the claim that increased levels of COX-2 in the tissue promote neoangiogenesis. COX-2 did not affect number of previously formed blood vessels visualized with CD34 and these results differ from some other conducted studies. For example, in the above mentioned study by Mao et al. CD34 was used to visualize MVD as well as in study by Sun et al. relatively Yu et al. (17-19).

Existence of statistically significant difference in expression of COX-2 protein between investigated and control group, and significant positive correlation with number of CD105 positive blood vessels confirmed our starting point that COX-2 promotes neoangiogenesis in gastric cancer. The differences observed in the studies may be attributed to the different methods of visualization since most studies used molecular methods of analysis. The study confirmed the existence of statistically significant higher expression of protein COX-2 in the gastric cancer samples compared to gastritis mucosa sample, and it was depended on the grade of tumor differentiation, relatively with highest expression in moderately differentiated tumor samples. The number of newly formed blood vessels in gastric cancer samples was higher when compared to gastritis samples but it did not demonstrate connection with the size of tumor or regional lymph node involvement. We did not establish any kind of connection between previously formed blood vessels visualized with CD34 and tumor size, relatively regional lymph node involvement. In our study we established statistically significant positive correlation between protein COX-2 expression and number of CD105 positive blood vessels but that type of connection was not found regarding expression of CD34.

Conclusions

Therefore we may conclude that COX-2 expression promotes neoangiogenesis in gastric cancer sample even though it did not show link with the tumor size or regional lymph nodes involvement in our study. What we hope to do in the future is to widen this investigation with additional markers and new techniques of investigation including introduction of molecular pathology methods.

References

1. *Folkman J. How is blood vessel growth regulated in normal and neoplastic tissue? Cancer Res. 1986; 46(2): 467-473.*
2. *Kumar V, Abbas A, Fausto N, editors. Robbins and Cotran Patologic Basis of Disease. 7th ed. Philadelphia: Elsevier Inc.; 2005.*
3. *Uefuji K, Ichikura T, Mochizuki H. Cyclooxygenase-2 Expression Is Related to Prostaglandin Biosynthesis and Angiogenesis in Human Gastric Cancer. Clin Cancer Res. 2000; 6: 135-138.*
4. *Wang D, DuBois R. Cyclooxygenase 2-derived prostaglandin E_2 regulates the angiogenic switch. PNAS 2004; 101(2): 415-416.*
5. *Perrone G, Santini D, Zagami M, Vincenzi B, Verzì A, Morini S, et al.COX-2 expression of ampullary carcinoma: correlation with different histotypes and clinicopathological parameters. Virchows Arch 2006; 449: 334-340.*
6. *Bakhle Y S. COX-2 and cancer: a new approach to an old problem. British Journal of Pharmacology. 2001; 134: 1137–1150.*
7. *Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994; 107(4): 1183-8.*
8. *Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. N Engl J Med 1991; 324: 1–8.*
9. *Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, et al. Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 1992; 84: 1875–87.*
10. *Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 1993; 143: 401–9.*
11. *Allen DC. Histopathology reporting: Guidelines for Surgical Cancer. 6th ed. London: Springer-Verlag; 2006.*
12. *Hamilton S R, Aaltonen L A, editors. World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Digestive System. IARC Press Lyon; 2000.*
13. *Ding S, Li C, Lin S, Yang Y, Liu D, Han Y et al. Comparative evaluation of microvessel density determined by CD34 or CD105 in benign and malignant gastric lesions. Human pathology. 2006; 37(7): 861-866.*
14. *Bialas M, Okon K, Czopek J. Assessing microvessel density in gastric carcinoma: a comparison of three markers. Pol J Pathol. 2003; 54(4): 249-252.*
15. *Samaka R, Abdou A, Abd El-Wahed M, Kandil M, El-Kady N. Cyclooxygenase-2 Expression in Chronic Gastritis and Gastric Carcinoma, Correlation with Prognostic Parameters. Journal of the Egyptian Nat. Cancer Inst. 2006 18(4): 363-374.*
16. *Sanz-Rodriguez F, Guerrero-Esteo M, Botella L M, Banville D, Vary C P H and Bernabe C. Endoglin Regulates Cytoskeletal Organization through Binding to ZRP-1, a Member of the Lim Family of Proteins. The Journal Of Biological Chemistry. 2004; 279(31): 32858–32868.*
17. *Mao X Y, Wang X G, Lv X J, Han C B. COX-2 expression in gastric cancer and its relationship with angiogenesis using tissue microarray. World J Gastroenterol. 2007; 13(25): 3466-3471.*
18. *Yu L Z, Gao H G, Bai J F, Sun G, Zhao H L, Sun L et al. Expression of COX-2 proteins in gastric mucosal lesions. World J Gastroenterol. 2004; 10(2): 292-294.*
19. *Sun W H, Sun L Y, Fang R N, Shao Y, Xu H C, Xue Q P et al. Expression of Cyclooxygenase-2 and Matrix Metalloproteinase-9 in Gastric Carcinoma and its Correlation with Angiogenesis. Japanese Journal of Clinical Oncology. 2005; 35(12): 707-713.*
20. *Ding S, Li C, Lin S, Yang Y, Liu D, Han Y et al. Comparative evaluation of microvessel density determined by CD34 or CD105 in benign and malignant gastric lesions. Human pathology. 2006; 37(7): 861-866.*

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Importance of angiogenesis as a prognostic parameter in colorectal adenocarcinomas

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Abstract

Objective: To evaluate the relationship of conventional prognostic factors and angiogenic factors in colorectal carcinomas and investigate the role of these parametres in the tumour biology.

Methods: In this retrospective study, immunohistochemical staining for vascular endothelial growth factor and basic fibroblastic growth factor was performed in 39 pathologically proven colorectal adenocarcinomas and neo-vasculature was counted to define microvessel density in order to evaluate the prognostic role of angiogenesis. The immunohistochemical staining of aforementioned markers in biopsy specimens and their relationship to other prognosic parametres were evaluated.

Results: This study suggested a significant correlation between the tumor stage and degree of differentiation and microvessel density. There was also a statistically significant correlation between vascular endothelial growth factor staining and microvessel density.

Conclusion: The current study suggests that, immunohistochemical staining for these growth factors in order to define the microvessel density of a tumor can be used as a prognostic marker only when it is used in combination with other prognostic markers. Further cases, are needed to define the role of angiogenesis which undoubtedly exist in the biology of colorectal adenocarcinomas.

Key words: Angiogenesis, bFGF, colorectal adenocarcinoma, microvessel density, VEGF.

Introduction

Angiogenesis is the formation of new blood vessels from the endothelium of the existing vasculature (1). When a new tumour reaches the size of 1–2 mm, its ulterior growth requires the induction of new blood vessel formation, which in turn may lead to the development of metastases. Angiogenesis is dependent on the balance between many stimulatory and inhibitory factors (2). Proangiogenic factors, such as vascular endothelial growth factor (VEGF), bind to sites on endothelial cells that lead to their proliferation (3). Microvessel density assessment is the most commonly used technique to quantify angiogenesis in cancer. It was first developed by Weidner et al (1991) and used as a panendothelial immunohistochemical marker for blood microvessels, mainly with Factor VIII related antigen (F. VIII Ag or von Willebrand's factor), CD31 or CD34, and rarely with CD105 (4, 5). Basic fibroblast growth factor (bFGF) belongs to the family of fibroblast growth factors (FGFs). The biologic activities of bFGF are mediated via the transmembrane FGFR of the tyrosine kinase receptor family. bFGF is involved in tumorigenesis and tumor growth via stimulating the proliferation of tumor cells and angiogenesis. It has been reported that in advanced colon cancer the expression of FGF and FGFRs are up-regulated in the tumor cells (6). Angiogenesis is an important process not only in tumor biology but for tumor prognosis as well (7). Several studies have been performed to evaluate angiogenesis and its relationship to prognosis in a variety of tumor types including lung, prostate, cervix, and liver carcinomas (8-11). Several growth factors have been identified that regulate angiogenesis in colorectal cancer; the most important of these being vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (bFGF) and various other angiogenic factors (12).

The aim of the present study is to evaluate the relationship of conventional prognostic factors

and angiogenic factors in colorectal carcinomas and investigate the role of these parametres in the tumour biology.

Methods

Thirty nine cases of classical adenocarcinomas of the colon diagnosed at the Pathology Department of Mersin University Medical School between the years 2000-2005 were included in the present study. The clinical follow-up data could not be obtained. The imaging and operation informations were obtained through case files and the macroscopic features of the tumors through pathology reports. The H&E stained sections were re-evaluated and histological evaluation and typing of the tumour were done according to WHO 2000 classification. Tumors were diagnosed as grade I in case of widespread gland formation, as grade II for tumors with less well defined glanduler structures, and as grade III in case of anaplastic single tumor cells. Angiolymphatic invasion and lymph node metastasis were evaluated for each case. Representative sections of tumor and normal tissue without necrosis were chosen and 5µm parafin sections from each case were prepared. Immunohistochemical staining was performed with a standart avidin-biotin-immunoperoxidase technique using antibodies to VEGF (Neomarkers, Ab-7, 1467-P0), bFGF (Santa Cruz, cat sc-79) and CD31 (Neomarkers, Ab, JC/70). As a positive control for VEGF and bFGF, cytoplasmic staining of invasive ductal carcinoma of the breast was used. For CD31, staining of vascular endothelium around the tumor was used as an internal control. The cytoplasmic staining of the invasive tumour cells were regarded as positive staining for each marker. The intensity of the staining for VEGF and bFGF were graded as: mild (+), moderate (++), and dense (+++) semiquantitatively. Micro-vessel density (MVD) measurement was performed as described by Weidner and colleagues (4) in tumour areas without necrosis, and distant from an ulcerated surface. 'Hot spot' areas with the most number of vessels were found for each case in 5 different fields under x100 magnification. In these areas, the number of CD31-stained vessels were counted under x400 magnification.

Data entry and analysis was performed using SPSS 10.0 software package. Descriptive statistics were used to summarize the data. Chi-square comparison of prognostic parameters, post-hoc T-test and Pearson correlation tests were used to compare the prognostic parametres. The 'p' value was used for significancy ($p<0.05$; significant, $p>0.05$; not significant).

Results

A total of 39 patients constituted the study group comprising of 14 women and 25 men. The mean age of all cases was ranging between 30-86 (64.38). Five of the tumors (12.8%) were located in the caecum, 4 (% 10.3) in right colon, 2 (5.1%) in transverse colon, 3 (7.7%) in left colon, 8 (38.5%) in sigmoid colon, 15 (20.5%) in rectum, and 2 (5.1%) in the rectosigmoid region. The average size of the tumors was 54.2 mm ranging between 15-130 mm. Among the 39 cases, 5 were (12.8%) stage 2, 17 were (43.6%) stage 3, and 17 (43.6%) were stage 4 according to the AJCC 2002 staging system. Twenty cases (48.8%) were grade 1 (well differentiated), 14 (38.4%) were grade 2 (moderately differentiated), and 5 (12.8%) were grade 3 (poorly differentiated) histopathologically. All 39 cases showed VEGF and bFGF immunostaining with varying intensities. Ten cases showed mild (+), 21 cases showed moderate (++) and 8 cases showed dense (+++) staining (Figure 1.a) with VEGF. The number of cases showing mild, moderate and dense staining (Figure 1.b) with bFGF were 6 (15.4%), 15 (38.5%), and 18 (46.1%), respectively. There was no statistically significant difference between the staining intensities of VEGF and bFGF (X^2 =3.188, p=0.527). When the VEGF staining intensity of the tumors were compared to their respective differentiation degrees, no statistically significant correlation was observed (X^2 =5.975, p=0.201). The same result was obtained for bFGF staining (X^2 =6.912, p=0.141). The VEGF staining intensities and MVD were also compared. An increase in the MVD was observed with increasing VEGF staining intensity. There was a statistically significant correlation between VEGF (+++) and VEGF (+) cases (Post hoc value=38.8250, p=0.001). The same correlation was observed between VEGF (+++) and VEGF (++) cases (Post hoc value=28.3393, p=0.005). However, there was no significant correlation between

VEGF (+) and VEGF (++) cases (Post hoc value=10.4857, p=0.238). There was no statistically significant correlation between bFGF staining intensity and MVD of the cases [(p=0.613, p=0.069). The MVD values were compared for different tumor grades. There was a statistically significant correlation between grade I and II tumors (Post hoc value=24.1357, p=0.006). There was not such a significant correlation between grade I and III tumors (Post hoc value=21.6500, p=0.77) and between grade II and III tumors (Post hoc value=2.4857, p=0.842). The metastatic (L1) and non-metastatic (L0) cases were compared for VEGF staining intensities. There was no significant correlation between L1 and L0 cases interms of VEGF staining intensity (X^2 =1.104, p=0.576). Same was true for bFGF staining intensity as well (X^2 =0.729, p=0.695). No significant difference was observed between the mean MVD values of L1 and L0 groups either (T test=0.126, p=0.900). The cases with (V1) and without (V0) vascular invasion were evaluated for VEGF staining. There was no statistically significant correlation between V1 and V0 groups in terms of VEGF staining intensity (X^2 =3.565, p=0.168). There was no statistically significant correlation between V1 and V0 groups in terms of bFGF immunoreactivity either (X^2 =2.127, p=0.345). The MVD values of V1 and V0 groups were also compared but no statistically significant correlation was observed (T test= 0.516, p=0.609) There was no significant correlation between MVD and mean tumor size either (Pearson correlation test=0.111, p=0.501).

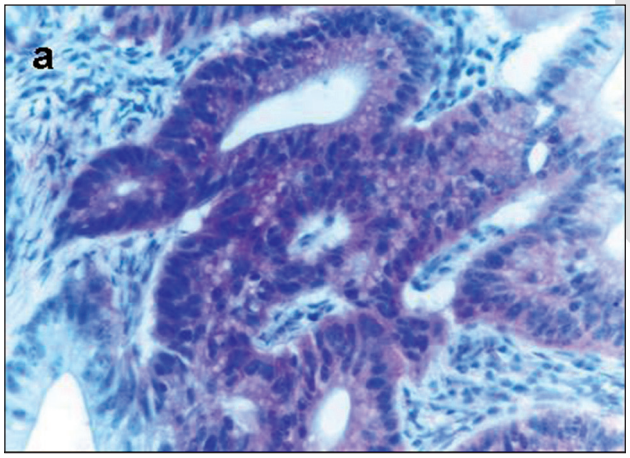

Figure 1.a VEGF staining of tumor cells; dense (+++), x400

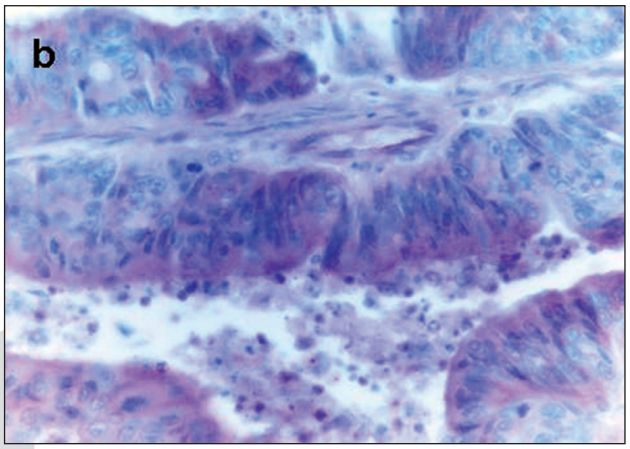

Figure 1.b. bFGF staining of tumor cells; dense (+++), x400

Discussion

Angiogenesis is a required process for tumour progression and metastasis. This process is dependent on the balance between the inhibitory and stimulating growth factors. This balance is disturbed in tumour kinetics and results in neovascularisation (13). Among many growth factors known to stimulate angiogenesis, VEGF is the one proved to have a major role in the process. VEGF is highly expressed in many of the primary and metastatic solid tumours of the gastrointestinal system (14). Several reports have proved a role of VEGF in proliferation, metastasis and angiogenesis in colon carcinomas (14, 15).

In the early phases of colon carcinoma FGF-BP is activated by bFGF and play a role in the formation of angiogenetic phenotype (16). The role of bFGF in the progression and invasion of colon carcinomas has been demonstrated in several in vivo and in vitro studies (17). In order to detect the level of angiogenesis, the newly forming vessels have to be measured in an experimental setting. Immunohistochemical analysis using antibodies directed against endothelial surface antigens (CD31, CD34, and Factor 8RA) combined with morphometric detection systems also give reliable results (18). It has been reported that the measurement of microvessel density (vessel/mm^2) in breast, lung, colon, stomach, cervix, head and neck, prostate and bladder carcinomas shows good correlation with pathological findings and gives prognostic information (4). Statistically significant correlation between differentiation of the tumours, prognosis, survival of the

patients and VEGF expression and angiogenesis has been shown for breast, brain, kidney, colon, ovary, cervix, head and neck tumours using molecular techniques (19). It has been shown in a study (6) designed to evaluate the prognostic role of angiogenesis in advanced stage lung cancer patients that patients with high level of neovascularisation had longer survival times with adjuvant therapy compared to patients with low level of neovascularisation. This study has also shown a correlation between high serum levels of VEGF and short survival times, poor prognosis and low response rate to therapy (8). In the present study, the MVD of stage II tumours were significantly higher than that of tumours in advanced stages. Furthermore, a positive correlation between VEGF staining intensity and MVD was observed. A prognostic conclusion could not be drawn from these results due to the lack of clinical follow-up data of the patients. Since there is no necrosis and hypoxic area in the tumour in the very early stages of the disease, the level of angiogenic factors produced by the tumour will be low which in turn will lead to a lower MVD. However, due to an inflammatory reaction to the tumour at these early stages of the disease, which leads to the production of an excess of angiogenic factors, angiogenesis might be induced. This seems to be the only plausible explanation to the positive correlation observed between high vessel number and longer survival. The most important prognostic factor in colorectal carcinomas is the stage of the tumour (20). An increase in the vascularity of the tumour increases the possibility of the tumour cells to enter the circulation and metastasize (21). Cascinu et al. showed that relapsing stage II colon carcinomas had a stronger VEGF expression compared to the nonrelapsing tumours, thus hypothesizing that VEGF could be a marker to define the cases with a high risk of relapse who would benefit from adjuvant therapy (22). In a study performed on stage II and III (n=131) colorectal carcinoma cases, Khorana et al. reported that VEGF was not expressed in 34% of the cases and that there was no correlation between the VEGF expression of tumour cells and the survival rates of the patients (23). In the current study there were no statistically significant difference between pT2, pT3 and pT4 cases in terms of VEGF and bFGF expression immunohistochemically. This contradicts with the results in the literature stating the increased production of angiogenic factors with increased stage. This may be due to the fact that there were pT1 cases in the current study as well as due to the fact that all cases were advanced stage cases with poor prognostic factors. This result may be improved in a study with more cases showing *homogeneous distribution* of tumour stage. In the present study VEGF and bFGF expression was detected in all cases immunohistochemically. It was not possible to reach the patients' clinical follow-up data in our study. So the parameters related to survival time could not be reviewed. The immunohistochemical results showed that cases with strong VEGF expression had a higher number of MVD as compared to cases with weak VEGF staining and cases with intermediate staining strength. There was a statistically significant correlation between VEGF expression of the tumour and MVD. This finding is in parallel with the reports (24) indicating that an increase in the production of angiogenic factors would lead to an increase in new vessel formation in colorectal carcinomas. However, a similar correlation could not be demonstrated between bFGF expression and MVD in the current study. This may be due to the fact that bFGF is a less potent angiogenic factor and stimulates angiogenesis using different pathways compared to VEGF. In a similar study performed by Takahashi et al., negative correlation was shown between MVD and prognosis and also between VEGF expression and survival in metastatic colon carcinomas (25). This further confirms the hypothesis that VEGF expression can be used as a marker to evaluate the metastatic potential of colorectal carcinoma. Weidner et al. showed a statistically significant relationship between increased MVD and tumour extension through the *capsule* in prostate cancer (26). In a similar study a statistically significant relationship between increased MVD and advanced disease, lymph node metastasis, relapse and survival has been reported for bladder carcinomas (27). We have evaluated MVD among different tumour grades which is an important prognostic parameter. The MVD of grade I tumours were higher than that of grade II tumours which was also statistically significant. This result may be due to the fact that the majority of the cases included in the current study were grade I tumours. In a study consisting of 22 colorectal carcinoma cases performed by Banner et

al. no significant relationship could be shown between MVD and survival, tumour localisation, lymphovascular invasion, tumour size, grade and the presence of musinous component (28). However, Saclarides et al. reported a significant relationship between MVD and tumour invasion and survival in a study consisting of 48 rectum carcinomas (29). Several similar studies in the literature also report significant relationship between MVD and tumour recurrence, survival, tumour invasion and haematogenous metastasis (30, 31, 32). There was no significant correlation between MVD, bFGF and VEGF expression and lymphovascular invasion and lymph node metastasis in our study. The secretion of growth factors is important but not sufficient for tumour metastasis. It is known that metastasis occurs via subclones in the tumours which gain metastatic potential. It maybe speculated that the results of the current study may reflect investigation of tumour subclones with no metastatic potential. It should also be considered that further sections obtained from nonmetastatic cases might reveal micrometastasis which would increase the number of early stage carcinomas with metastatic properties which in return could result in statistically significant results. All of the above mentioned studies report a significant correlation with increased MVD and aggressive clinical behaviour. However, in a study consisting of 227 colorectal carcinomas performed by Lindmark et al., statistically significant correlation was detected between MVD and survival, whereas no significant correlation was observed between MVD and Dukes stage and tumour differentiation (33). In the present study the MVD values of pT2, pT3 and pT4 tumour groups were 94, 67, 70 vessel/mm^2, respectively. There was a statistically significant relationship between MVD values and stage. However in contrast to most of the similar studies, there was a negative correlation between both pT2 and pT3 groups and MVD (p=0.043). This may again be due to the small size of the pT2 group. Several studies investigating tumour biology have shown that tumours that are 2-3 mm in diameter get their nutritious supply via diffusion from the surrounding vessels, whereas further growth requires formation of new vessels. In an experimental study it has been shown that tumour tissues placed to the anterior avascular part of bulbus oculi did not grow whereas tumours placed to the posterior vascular part have grown to fill the orbital cavity (34). The mean tumour size in this study was 54.2 mm. There was no significant correlation between tumour size and MVD. However, the tumours included in the study were all large tumours and inclusion of tumours with various sizes may lead to a significant correlation.

Angiogenesis plays an essential role during embryogenesis, adult physiological vascular re-modeling, some pathological events such as diabetic retinopathy and sometimes in tumor development (35). Both clinical and experimental data show that most human tumours during the earlier phase of their growth do not cause new vessel formation. Tumours remain in situ for a long time without developing new blood supply, and probably gain angiogenic properties due to accumulating mutations. Although the molecular basis of gaining angiogenic properites is not yet well understood, it is due to increased production of angiogenic factors or loss of inhibitors of angiogenesis (36).

In conclusion, VEGF and bFGF expression patterns maybe included in the clinicopathological evaluation process of colorectal carcinomas in addition to the standard prognostic criteria. However, in order to define the exact role of expression of VEGF and bFGF on patient survival, studies performed on larger case numbers and on tumours with different stages with clinical follow-up data are required.

References

1. *Pandya NM, Dhalla NS, Santani DD. Angiogenesis-a new target for future therapy. Vascul Pharmacol 2006; 44: 265-74.*

2. *Sampaio PC, Auslaender D, Krubasik D, Failla AV, Skepper JN, Murphy G, English WR. A Heterogeneous In Vitro Three Dimensional Model of Tumour-Stroma Interactions Regulating Sprouting Angiogenesis PLoS One. 2012; 7: 2.*

3. *Kerbel RS. Tumor angiogenesis: past, present and the near future. Carcinogenesis 2000; 21(3): 505-515.*

4. *Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med. 1991 3; 324(1): 1-8.*

5. *Zhang YY, Sun CF, Peng J. Expression of CD31, CD34 and CD105 in squamous cell carcinoma of tongue and their relationships with lymph node metastasis. Hua Xi Kou Qiang Yi Xue Za Zhi 2008; 26(2): 201-5.*

6. *Wang et al. Mechanism of antitumor effect of a novel bFGF binding peptide on human colon cancer cells. Cancer Sci. 2010; 101 (5): 1212-18).*

7. *Liang JF, Wang HK, Xiao H, Li N, Cheng CX, Zhao YZ, Ma YB, Gao JZ, Bai RB, Zheng HX. Relationship and prognostic significance of SPARC and VEGF protein expression in colon cancer. J Exp Clin Cancer Res. 2010; 16; 29: 71.*

8. *Salven P, Ruotsalainen T, Mattson K, Joensu H. High pretreatment serum level of vascular endothelial growth factor (VEGF) is associated with poor outcome in small-cell lung cancer. Int J Cancer. 1998; 79: 144-146.*

9. *Harper ME, Glynne JE, Goddard L, Thurston VJ, Griffiths K. Vascular endothelial growth factor (VEGF) ekspression in prostatic tumors and its relationship to neuroendocrine cells. BR J Cancer. 1996; 74: 910-916.*

10. *Obermair A, Bancher-Todesca D, Bilgi S, Kaider A, Kohlberger P, Müllauer-Ertl S, Leodolter S, Gitsch G. Correlation OF Vascular endothelial growth factor expression and microvessel density in cervical intraepithelial neoplasia. J Natl Cancer Inst. 1997; 89: 1212-1217.*

11. *Torimura T, Sata M, Ueno T, Kin M, Tsuji R, Suzaku K, Hashimoto O, Sugawara H, Tanikawa K. İncreased expression of vascular endothelial growth factor is associated with tumor progression in hepatocellular carcinoma. Hum Pathol. 1998; 29: 986-991.*

12. *Narita K, Fujii T, Ishiwata T, Yamamoto T, Kawamoto Y, Kawahara K, Nakazawa N, Naito Z. Keratinocyte growth factor induces vascular endothelial growth factor-A expression in colorectal cancer cells. Int J Oncol. 2009; 34(2): 355-60.*

13. *Takahashi Y, Bucana CD, Cleary KR, Ellis LM. P53, vessel count and vascular endothelial growth factor expression in human colon cancer. Int J Cancer. 1998; 79: 34-38.*

14. *Takeda A, Stoeltzing O, Ahmad SA, Reinmuth N, Liu W, Parikh A, Fan F, Akagi M, Ellis LM. Role of angiogenesis in the development and growth of liver metastasis. Ann Surg Oncol. 2002; 9(7): 610-6.*

15. *Ellis LM, Takahashi Y, Liu W, Shahen RM. Vascular endothelial growth factor in human colon cancer: biology and therapeutic implications. The Oncologist. 2000; 5: 11-1.*

16. *Ray R, Manzano RC, Moser AR, Waldman T. Up-regulation of fibroblast growth factor-binding protein by-beta catenin during colon carcinogenesis. Cancer Research. 2003; 63: 8085-8089.*

17. *Jayson GC, Vives C, Paraskeva C. Coordinated modulation of the fibroblast growth factor dual receptor mechanism during transformation from human colon adenoma to carcinoma. Int J Cancer. 1999; 82: 298-304.*

18. *Folkman J. The role of angiogenesis in tumor growth. Cancer Biol. 1992; 3: 65.*

19. *Kajdaniuk D, Marek B, Foltyn W, Kos-Kudła B. Vascular endothelial growth factor (VEGF) - part 2: in endocrinology and oncology. Endokrynol Pol. 2011; 62(5): 456-64.*

20. *Rosai J. Rosai and Ackerman's Surgical Pathology, 9th ed. St Luis : Mosby, 2004; 1, pp. 811-821.*

21. *Weidner N, Folkman J. Tumoral vascularity as a prognostic factor in cancer. Important Adv Oncol. 1996: 167-90.*

22. *Cascinu S, Stacciolli MP, Gasparini G, Giordani P. Expression of vascular endothelial growth factor can predict event free survival in stage II colon cancer. Clinical Cancer Research. 2000; 6: 2803-28*

23. *Khorana AA, Ryan CK, Cox C, Eberly S, Sahasrabudhe DP. Vascular endothelial growth factor, CD68, epidermal growth factor receptor expression and survival in patients with stage 2 and 3 colon carcinoma. Cancer. 2003; 97: 960-968.*

24. *Liang J, Wang H, Xiao H, Li N, Cheng,C, Zhao Y, Ma Y, Gao J, Bai R, Zheng H. Relationship and prognostic significance of SPARC and VEGF protein expression in colon cancer. J Exp Clin Cancer Res. 2010; 29(1): 71.*

25. *Takahashi Y, Tucker SL, Kitadai Y. Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node negative colon cancer. Arch Surg. 1997; 132: 541-546.*

26. *Weidner N, Caroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenezis correlates with metastasis in invasive prostat carcinoma. American J Pathol. 1993; 143: 401.*

27. *Hawke CK, Delahunt B, Davidson PTJ. The prognostic value of microvessel density in bladder cancer. J.Urol. 1995; 154; 62.*

28. *Banner BF, Whitehouse R, Paker SP, Swanson RS. Tumor angiogenesis in stage 2 colorectal carcinoma. Am J Clin Pathol. 1998; 109(6): 733-737.*

29. *Saclarides TJ, Speziale NJ, Drab E. Tumor angiogenesis and rectal carcinoma. Dis colon Rectum. 1994; 37: 921-926.*

30. *Frank RE, Saclarides TJ, Leurgans S, Speziale NJ, Drab EA, Rubin DB. Tumor angiogenesis as a predictor of recurrense and survival in patients with node negative colon cancer. Ann Surg. 1995; 222: 695-699.*

31. *Engel CJ, Bennett ST, Chambers AF, Doig GS, Kerkvliet N, O'Malley FP. Tumor angiogenesis predicts recurrence in invasive colorectal cancer when controlled for Dukes staging. Am j Surg Pathol. 1996; 20: 1260-1265.*

32. *Tanigawa N, Amaya H, Matsumura M, Lu C, Kitaoka A, Matsuyama K, Muraoka R. Tumor angiogenesis and mode of metastases in patients with colorectal cancer. Cancer Res. 1997; 57: 1043-1046.*

33. *Lindmark G, Gerdin B, Sundberg C, Påhlman L, Bergström R, Glimelius B. Prognostic significance of microvascular count in colorectal cancer. J Clin Oncol. 1996; 14: 461-466.*

34. *Folkman J. The role of angiogenesis in tumor growth. Cancer Biol. 1992; 3: 65.*

35. *Altuntaş EE, Balaban H, Uysal İÖ, Akın PZ. The Effect of Pentoxiphylline, a non-specific phospodiesterase inhibitor, on Angiogenesis in Chicken ChorioallantoicMembrane (CAM) Model. HealthMED. 2011; 5: 1841-1846.*

36. *Calvo A, Yokoyama Y, Smith LE, Ali I, Shih SC, Feldman AL, Libutti SK, Sundaram R, Green JE. Inhibition of the mammary carcinoma angiogenic switch in C3(1)/SV40 transgenic mice by a mutated form of human endostatin. Int J Cancer. 2002; 20; 101(3): 224-234.*

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Sexual behaviours toward sexually transmitted infections: A cross-sectional survey among undergraduate medical students

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Abstract

Introduction: Sexually transmitted infections (STIs) are global healthcare problem today and the most spread infectious diseases. The main purpose of this study was to assess whether STI risky-taking behaviours among undergraduate medical students in BaH, canton Sarajevo, are related to each other and what are the personal, family or social/school-related factors influencing them.

Methods: A cross-sectional study was conducted among two groups of 188 and 230 students aged 18-25 and ≥26 by means of self reported questionnaire forms at the University of Sarajevo, BaH. Data were collected in December-January 2011/2012 and in 2012/2013.

Results: Overall, 188 participants in the first part of the survey completed the questionnaire forms of whom 47 (35.3%) males and 86 (64.7%) females, aged 17-21. The second aged group 22-25, conducted 28 (50.9%) males and 27 (49.1%) females (p=0.47). In the second part of the survey 230 undergraduate medical students were observed in the analyzed sample, divided into three age groups. In the group 17-21 of age, 28 (30.4%) males and 64 (69.6%) females were examined. The second age group 22-25, conducted 34 (39.1%) males and 53 (60.9%) females, respectively. Among student population aged ≥ 26 years, 24 (47.1%) males and 27 (52.9%) females were observed (p=0.132). Variables of interest included age, gender, sexual behaviour and sexual experience, history of STIs, condom use and living with both parents until 18 years of age. According to questionnaire form in the first part, sexual experience have had 127 (67.5%) of students, out of which 68 (53.5%) males and 59 (46.5%) females, (p=0.000). In the second part, sexual experience have had 131 (56.59%) of students, out of which 72 (55.0%) males and 59 (45.0%) females (p=0.000).

Conclusions: The key findings indicate that most university students are involved in risky sexual undertakings. Risky sexual behaviours is influenced by gender, sexual expereince, first sexual intercourse, condom use and lifetime number partners.

Key words: Sexually transmitted infections, sexual behaviour, medical students.

Introduction

Sexually transmitted infections (STIs) are global healthcare problem today and the most spread infectious diseases. Their incidence is constantly increasing, primarily affecting adolescents and young adults (1). The World Health Organization (WHO) estimates that approximately 448 million new cases of curable STIs (243 million males and 204 million females) occur each year, 75-85% of them in developing countries (2). The youth represents the most vulnerable population and bears much of the burden of STIs. Nowdays, morbidity and mortality among youth is mainly due to their lifestyles-derived from risky behaviours. The risky behaviours could negatively affect health, such as, among other things, drug use, unsafe sexual practices, or violent behavior. The potentially negative consequences of these behaviours include unintended pregnancy, aquisition of STIs (including HIV infection), infertility, pelvic inflammatory disease (PID), and death as well (3). There are many factors that have beeen described in the literature that can influence risky-taking behaviours. These are mainly personal, fam-

ily or social/school-related (4,5). Some authors emphasize that the changes in social context are the main reason why youth begin with sexual activities at their earlier age (6). Therefore, the use of condom by young men must be considered as an important and urgent issue (7). There are important differences between males and females attitudes about sexuality in BaH. According to literature the first sexual experinece happens at the age of 15 in 39.9% female adolescents and 34.9% male adolescents in England, 47.1% male adolescents in Ukraine and 45.2% in Slovenia, and 38.5% female adolescents in Wales (8).

In Bosnia and Herzegovina, as in the other countries of Eastern Europe, there is a lack of data on prevalence of the most curable STIs in the general population, including youth (9).

The main purpose of this study was to assess whether STI related risky-taking behaviours among undergraduate medical students aged 18-25 and ≥26 in BaH, canton Sarajevo, are related to each other and what are the personal, family or social/school-related factors influencing them.

In addition, this study aimed to explore and propose recommendation for future interventions, and provide evidence for implementing education strategies to present STI/HIV among youth in BaH.

Material and methods

A cross-sectional study was conducted among 188 and 230 medical students by means of self reported questionnaire forms at the University of Sarajevo, BaH. We included undergraduate medical students, both sexes, aged 18-25 and ≥26 in canton Sarajevo. Data were collected in December-January 2011/2012 and in 2012/2013. The survey was conducted among students of different faculties, departments and academic years. Examinees was chosen randomly, with different places and way of living (with parents, on their own, in a dormitory), from different living and working environments and different in religious and ethnic affiliation. A self administered, Bosnian language questionnaire form consisted of three parts, including 37 questions, was developed for the study. The first part comprised of 7 items; general epidemiological data, demographic and socio-economic questions of respondents as well. The second part contained 6 items related to knowledge towards STI/HIV. The third part contained 24 items related to sexual behaviours and sexual experience, history of sexually transmitted infections, belifies about condom and condom use. The survey questionnaire form was designed in reference to the questionnaires of sexual knowledge, sexual attitudes, and sexual behaviours of the WHO, as well as other relevant documents and literature. The questionnaire form was answered within 15-20 minutes. We conducted this study in accordance with ethical principles of the Declaration of Helsinki. The data were collected with questionnaires form prepared by the researchers. Standard methods of descriptive and inference statistics were applied. All data was anonymous and confidential. Analysis data was performed using SPSS softwer version (SPSS, Chicago, IL) 17. for Windows. Level of significance $p<0.05$ was used in testing of statistical hypothesis.

Results

Overall, 188 participants in the first part of the survey completed the questionnaire forms of whom 47 (35.3%) males and 86 (64.7%) females, aged 17-21. The second aged group 22-25, conducted 28 (50.9%) males and 27 (49.1%) females, statistically independent (p=0.47) (Table 1.). Variables of interest included age, gender, sexual behaviour and sexual experience, history of STIs, condom use and living with both parents until 18 years of age. In the second part of the survey 230 undergraduate medical students were observed in the analyzed sample, divided into three age groups. In the group 17-21 of age, 28 (30.4%) males and 64 (69.6%) females were examined. The second age group 22-25, conducted 34 (39.1%) males and 53 (60.9%) females, respectively. Among student population aged ≥ 26 years, 24 (47.1%) males and 27 (52.9%) females were observed (p=0.132). According to questionnaire form in the first part, sexual experience have had 127 (67.5%) of students, out of which 68 (53.5%) males and 59 (46.5%) females, (p=0.000). In the second part, sexual experience have had 131 (56.59%) of students, out of which 72 (55.0%) males and 59 (45.0%) females (p=0.000). The first sexual intercourse at the age ≤16 have had 20 (87.0%) males and 3 (13.0%) females, as well as at the age ≥17, 48 (46.2%) males and 56 (53.8%) females in the first

part, (p=0.000). In the second part, the first sexual intercourse at the age ≤16 have had 20 (90.9%) males and 2 (9.1% females), as well as at the age of ≥17, 47 (45.2%) males and 57 (54.8%) females, p=0.000). The variable, lifetime number partners in the past 12 months, 0-1 have had 48 (37.8%) males and 79 (62.2%) females and ≥2 have had 22 (88.0%) males and 3 (12.0%) females in the first part, (p=0.000), while in the second part, 0-1 have had 38 (49.9%) males and 43 (53.1%) females, ≥2 have had 22 (62.9%) males and 13 (37.1%) females (p=0.169). Following variable, life time partners, according to gender, 0-1 partner in the first part have had 25 (33.3%) males and 71 (62.2%) females; 2-3 have had 17 (65.4%) males and 19 (34.6%) females and ≥4, 28 (96.6%) males and 1 83.4%) females, respectively. The same variable 0-1 in second part have had 12 (26.7%) males and 33 (73.3%) females; 2-3 13 (46.4%) males and 15 (53.6%) females and ≥4, 28 (82.4%) males and 6 (17.6%) females (p=0.000). Condom used at last intercourse in the first part have had 31 (64.6%) males and 17 (35.4%) females (p=0.000), in the second part, 33 (54.1%) males and 28 (45.9%) females (p=0.796). Permanent partner in the first part have had 31 (64.6%) males and 17 (35.4%) females ((p=0.000); in the second part, 45 (47.9%) males and 49 (52.1) females (p=1.000). The analyzed variable, life with both parents until 18 years old, in the first part have had 62 (38.5%) males and 99 (61.5%) females (p=0.463); in the second part, 71 (37.4%) males and 119 (62.6%) females (p=0.951). Table 2. showed the items associated to history of STIs according to gender as well. The variable, history of STIs, in the first part have had 1 (33.3%) males and 2 (66.7%) females (p=1.000); in the se-

Table 1. Items associated to sexual behaviour according to gender

	2011/12 (n=188)			2012/13 (n=230)		
	Male	**Female**	**P-value**	**Male**	**Female**	**P-value**
Age group						
17-21 22-25 ≥26	47 (35.3%) 28 (50.9%)	86 (64.7%) 27 (49.1%)	0.069***	23 (30.4%) 34 (39.1%) 24 (47.1%)	64 (69.6%) 53 (60.9%) 27 (52.9%)	0.132*
The sexual intercourse ever						
Yes No	68 (53.5%) 7 (11.8%)	59 (46.5%) 54 (88.2%)	0.000**	72 (55.0%) 12 (13.6%)	59 (45.0%) 76 (86.4%)	0.000***
Age of first sexual intercourse						
≤16 ≥17	20 (87.0%) 48 (46.2%)	3 (13.0%) 56 (53.8%)	0.000**	20 (90.9%) 47 (45.2%)	2 (9.1%) 57 (54.8%)	0.000**
Lifetime number partners in the past 12 months						
0-1 ≥2	48 (37.8%) 22 (88.0%)	79 (62.2%) 3 (12.0%)	0.000*	38 (49.9%) 22 (62.9%)	43 (53.1%) 13 (37.1%)	0.169***
Lifetime number partners						
0-1 2-3 ≥4	25 (33.3%) 17 (65.4%) 28 (96.6%)	71 (74.0%) 19 (34.6%) 1 (3.4%)	-	12 (26.7%) 13 (46.4%) 28 (82.4%)	33 (73.3%) 15 (53.6%) 6 (17.6%)	0.000*
Condom used at last intercourse						
Yes No	34 (59.6%) 41 (31.3%)	23 (40.4%) 90 (68.7%)	0.000***	33 (54.1%) 28 (50.0%)	28 (45.9%) 28 (50.0%)	0.796***
Permanent partner						
Yes No	31 (64.6%) 44 (31.4%)	17 (35.4%) 96 (68.6%)	0.000***	45 (47.9%) 26 (48.1%)	49 (52.1%) 28 (51.9%)	1.000***
Life with both parents until 18 years of age						
Yes No	62 (38.5%) 13 (48.1%)	99(61.5%) 14 (51.9%)	0.463***	71 (37.4%) 15 (39.5%)	119 (62.6%) 23 (60.5%)	0.951***

Pearson Chi-Square **Fisher's Exact Test * Continuity Correction*

cond part, 3 (75.0%) males and 1 (25.0%) females, respectively. As it showed in table 2. following etiologic agents have been documented: *Candida spp.*, *Trichomonas vaginalis*, *N. gonorrhoeae*, *Herpes simplex* type *2,* human papillomavirus. The most prevalent STI symptoms included: vaginal discharge, in 16 (10.0%) females, abdominal pain, in 11 (84.6%) females, (p=0.042) and bleeding, in 4 (100.0%) males, (p=0.032).

Discussion

Sexually transmitted infections are unique in infectious disease category, bearing in mind that they are completely dependent on behaviuoral factors for transmission. These kinds of infections also present numerious barriers to routine clinical care and diagnostics because of the associated stigma, costs and confidentiality issues. Youth represents the most vulnerable population and bears much of the burden of sexually transmittes diseases (STDs). Studies indicate that one in four sexually active adolescents will acquire an STDs (10,11). Many adolescents, and even clinicians are reluctant to discuss sexual health issues openly because of the social implications, issues of privacy and confidentiallity (12). Assesing sexual behaviour is critical to the development of behaviour and disease control interventions. In the USA, a number of large cross-sectional national surveys have been very useful in

Table 2. Items associated to history of STIs according to gender

	2011/12 (n=188)			2012/13 (n=230)			
	Male	**Female**	**P-value**	**Male**		**Female**	**P-value**
History of STIs							
Yes No Don´t know	1 (33.3%) 74 (40.0%)	2 (66.7%) 111 (60.0%)	1.000**	3 (75.0%) 74 (37.8%) 2 (50.0%)		1 (25.0%) 122 (62.2%) 2 (50.0%)	-
Etiologic agents related to STIs							
Candida spp. *Trichomonas v.* Gonorrhoea Herpes Human papillomavirus	1(100.0%) 0 (0.0%) - - -	0 (0.0%) 1 (100.0%) - - -	-	- - 1 (100.0%) 1 (100.0%) 1 (50.0%)		- - 0 (0.0%) 0 (0.0%) 1 (50.0%)	-
STIs therapy							
Yes No	2 (50.0%) 0 (0.0%)	2 (50.0%) 0 (0.0%)	-	4 (80.0%) 0 (0.0%)		1 (20.0%) 0 .(0.0%)	-
Symptoms related to STIs							
Urethral discharge	1 (100.0%)	0 (0.00%)		Yes No	2 (50.0%) 51 (41.8%)	2 (50.0%) 71 (58.2%)	1.000**
Vaginal discharge	0 (0.00%)	3 (100.0%)		Yes No	0 (0.00) 48 (43.6%)	16 (10.0%) 62 (56.4%)	-
Difficult and painful urination	1 (50.0%)	1 (50.0%)	-	Yes No	1 (50.0%) 52(41.9%)	1 (50.0%) 72 (58.1%)	1.000**
Itching	1 (33.3%)	2 (66.7%)		Yes No	3 (50.0%) 50 (41.7%)	3 (50.0%) 70 (58.3%)	0.695**
Bleeding	–	–		Yes No	4 (100.0%) 51 (41.1%)	0 (0.0%) 73 (59.9%)	0.032**
Abdominal pain	–	–		Yes No	2 (16.4%) 51 (44.7%)	11 (84.6%) 63 (55.3%)	0.042**
None of the above	–	–		Yes No	19 (31.0%) 15 (55.6%)	42 (68.9%) 12 (44.4%)	0.053***
Others	–	–		Yes No	-	-	-

Pearson Chi-Square **Fisher's Exact Test * Continuity Correction*

assesing behaviour and trends. These have included Sexual Behaviour, Sexual Attraction and Sexual Identity in the USA: data from 2006-2008 National Survey of Family Growth and the Youth Risk Behavioural Surveillance (13,14).

Late adolescence is a time of self-exploration and identity development, which includes sexual identity formation (15,16). College life provides a sense of independance and pressure for sexual experimentation. Youth of college age are at high risk for STIs. An estimated 19 million STIs occur annually with almost half occuring among 15-24 year olds (14). Young people aged 15-24 years also have four times the reported chlamydia and gonnorhea rates of the total population aged 10-65+ years (14). Moreever, adolescents and young adults aged 13-29 years accounted for 39% of new HIV infections in 2009 (14).

According to questionnaire, our results showed that tested students have had the first sexual intercourse oftenly in age group ≥ 17, mostly females (53.8% vs. 54.8%), (p=0.000), which is in concordance with previously conducted research among young people in canton Tuzla (17). Data from 2007 in Croatia has shown that 18.6% of boys and 16.5% of girls had their first sexual experience by the age of 16 (18). It is known that 15-24 age group posses greater risks for STIs and HIV wordwide (19). Higher proportions of male university students were sexually active than female students this fact was consistent with a number of other studies done in different parts (20, 21, 22). Results from our study confirmed the same results (55.0% vs.45.0%), (p=0.000). For instance, a survey done in Gondar College of medical science (20), [55.2% vs. 8.3%], a study conducted among university students in China (21), where 17.6% of males and 8.6% of females were sexually active. Another study done in Haramaya University (22) showed similar evidence [59.4% vs. 38.9%].

The impact of family and school on adolescent health has been shown repeatedly by studies in North America and Western Europe (19, 23). Studies in North and South America have shownstrong associations between adolescent sexual behavIour and interpersonal family realtionships, the education, presence, and expectations of parents, and the connectdness of the adolescents to parents and school (24). Additionally, there is evidence in developed and developing countries that knowledging the sexual acitivity of adolescents and meeting their sexual health needs with targeted education and preventive care services can help reduce risky sexual behaviour and its consequences (24, 26). According the variable life with parents until 18 years of age, our results showed that females mostly living with parents (61.5% vs. 62.6%) than males.

Condoms are effective when used correctly and consistently in reducing the risk of STIs, including HIV (26). Our investigation confirmed that in the first part of the survey there were statistically significant dependence according to condom use and gender. Findings from University in Yozgat, Turkey, confirmed that male university students who are active generally do not widely used condom sufficiently (27). In other studies, it was found that most of the male students had sexual experience without proper protection methods (28, 29). It was found that majority of the young males aged 20 had sex with more than one partner (30, 31), which is similar to our results.

The key findings indicate that most university students are involved in risky sexual undertakings. Risky sexual behaviours is influenced by gender, sexual expereince, first sexual intercourse, condom use and lifetime number partners.

Health education of youth improves their knowledge of sexual behaviour, provides support and develops skills needed for avoiding risky-taking behaviours, as well as preventing major STIs.

References

1. *Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2009. Atlanta, GA: U.S. Department of Health and Human Services, 2010. CDC, 2010.*
2. *WHO. Global prevalence and incidence od selected curable sexually transmitted infections: overviews and estimates. WHO/HIV_AIDS/2005.Geneva: WHO, 2005.*
3. *Igra V, Irwin CE. Theories of adosecent risk-taking behaviour. In: DiClemente RJ, Hansen WB, Ponton Le, eds. Handbook of adolescent health risk behaviour. New York: Plenum Press 1996.*
4. *Kuperman S, Schlosser SS, Kramer JR, Bucholz K, Hesselbrock V, Reich T et al. Risk domains associated with an adolescent alcohol dependence diagnosis, Adiction 2001; 96: 629-36.*

5. *Gil AG, Vega WA, Turner RJ. Early and mid-adolescence risf factors for later substance abuse by African Americans and European Americans. Public Health Rep 2002; 117 (Supple 1): S 15-29.*
6. *Stulofer A. Sociolcultural and psycho-social aspects of risk sexual behaviour. Medicus 2009; 18 (suppl 1): 123-9.*
7. *Aras S, Semin S, Gunay T, Orcin E. Ozan S. Sexual attitudes and risk-taking behaviours of high school students in Turkey. Journal of School Health 2007; 77 (7): 359-366.*
8. *9. Chapman JR. Adolescent sex and mass media. Adolescence Winter 2000; 140: 799-811.*
9. *Uuskula A, Puur A, Toompere K, DeHovitz J: Trends in the epidemiology of bacterial sexually transmitted infections in Eastern Europe, 1995-2005. Sex Trans Infect 2010; 86: 6-14.*
10. *Chesson HW, Bianford JM, Gift TL, Harrell W, Tao G, Irwinet KL. The estimated direct medical cost of sexually transmitted diseseases among American youth, 2000. Perspect Sex Reprod Health 2004,36: 11-19.*
11. *Hall HI, Riuguang S, Rhodes P, Prejean J, Qian A, Lee LM, et al. Estimation of HIV incidence in the United States. JAMA 2009; 300: 520-9.*
12. *Nurutdinova D, Rao S, Shacham E, Reno H, Overton ET. STD/HIV risk among adults in the primary care setting: are we adequately addresing our patients needs? Sex Trans Dis 2010; 38: 30-2.*
13. *Centers for Disease Control and Prevention. Sexual behaviour, sexual attraction, and sexual identity in the United States: data from 2006-2008 National Survey Report 2011; 36: 1-36.*
14. *Centers for Disease Control and Prevention. Surveillance Summeries. Sexual identity, sex of sexual contacts, and health-risk behaviours among students in grade 9-12 –Youth risk behavour surveillance, Selected Sites, United Stattes, 2001-2009. MMWR Surveill Summ 2011; 60: 1-133.*
15. *Erik H. Erikson. Identity, youth and crisis. New York: W. W. Norton Company, 1968.*
16. *Moore S, Rosenthal D. Sexuallity in adolescence. Current trends. London, UK. Taylor and Francis, 2006.*
17. *Hadzimehmedovic A, Balic A, Balic D. First sexual intercourse (sexarche) in youth in Bosnia and Herzegovina. Med Glas Ljek komore Zenicko-doboj kantona 2012; 9(1): 74-78.*
18. *Kuzman M, Pavic Simetion I, Pejnovic Franelic I. Early sexual intercourse and risk factoris in Croation adolescents. Coll Antropol 2007; 31 (suppl 2): 121-30.*
19. *Blum RW, Nelson MR. The Health of young people in a global context. J Adolescent Health 2004; 35: 402-418.*
20. *Fitaw Y, Worku A: High risk sexual behaviour and pattern of condom utilization of the Gondar College of medical Sciences students, North Western Ethiopia. Ethiop J Health Dev 2002; 16 (3): 335-338.*
21. *Qiaoqin M, Masako O, Liming C: Sexual behaviour and awerness of Chinese university students in transition with implied risk of sexually transmitted diseases and HIV infection. A cross-sectional study. BMC Publ Health 2006; 232 (6): 1471-2458.*
22. *Negash B: Predictors of condom use by using heath model among Haramaya University Students. AAU unpublished MPH Thesis. 2005.*
23. *Brieger WR, Delano GE, Lane CG, Oladepo O, Oyediran KA. West African Youth Initiative: outcome of a reproductive health education program. J Adolesc Health 2001; 29: 436-46.*
24. *Allan Guttmacher Institute. Can more progress be made? Teenage sexual and reproductive behaviour in developed countries (executive summary). Washington, DC. Allan Guttmacher Institute, 2001.*
25. *Kirby D. Emerging answers: research findings on programs to reduce teen pregnancy (summary). Washington, DC. National Compaign to prevent Teen Pregnancy; 2001.*
26. *Wald A, Langeberg AG, Link K, Izu AE, Ashley R, Warren T, Tyring S, Douglas JM Jr, Corey. Effect of condoms on reducing the transmission of herpes simplex virus type 2 from men to women. JAMA 2001: 285: 3100-6.*
27. *Polat S, Yuzer S, Baser M. Turkish male university students knowledge and attitudes about use of condom. HealthMED 2011: 5 (3): 633-638.*
28. *Aras S, Orcin E. Ozan S, Semin S. Sexual behaviours and contraception among university students in Turkey. J Biosoc Sci ... SAHARA J. 2008 Apr; 5(1): 28-35.*
29. *Karaduman F, Terzioglu F. Knowledge And Practice Regarding Emergency Contraception. Journal of Medical Sciences. 2008; 28 (6): 899-908.*
30. *Youth and sexuality. htpp: //www.cetad.org.tr/duyuru.php?id=17, December 2007.*
31. *Rotermann M. Sex, condoms and STDs among young people. Health reports (Statistics Canada, Catalogue 82-003) 2005; 16(3): 39-45.*

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Importance, knowledge, and behavior of mobbing in family physicians

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Abstract

Objective: In this study, we aimed to evaluate the importance, knowledge, and behavior of psychological abuse in the form of mobbing perpetrated by or to family physicians during their healthcare services.

Material and Method: Our study sample consisted of a total of 130 family physicians working in Malatya, a province of Turkey, in November 2012. However, while 120 physicians agreed to participate in the study, 10 did not. The study was performed by using a questionnaire. The responses to this questionnaire were analyzed by SPSS 15.0 package program using Pearson's chi-squared, Yates corrected chi-squared, and Fisher's exact tests. $P<0.05$ was recognized as statistically significant.

We applied multivariate logistic regression analysis to determine the parameters that may be associated with mobbing (age, gender, marital status, administrative responsibility, knowing the meaning of mobbing, describing mobbing as a malevolent behavior, working years).

The goodness of fit for the estimated multivariate logistic regression model was measured with Hosmer and Lemeshow test.

Conclusion: The most influential parameters in mobbing were found to be having an administrative responsibility (OR=16.4; 95% CI=1.24-216.5) and working years (OR=6; 95% CI=1.21-29.3). Based on this analysis, the most important factor in mobbing appears to be having an administrative responsibility.

The goodness of fit for the estimated multivariate logistic regression model was measured with Hosmer and Lemeshow test. The model was found to have a good degree of goodness of fit (chi-squared=2.43, df=7, P=0.93).

Key Words: Healthcare service, family physician, mobbing.

Introduction

In this study, we aimed to evaluate the importance, knowledge, and behavior of psychological abuse in the form of mobbing perpetrated by or to family physicians during their healthcare services.

Mobbing is an act of emotional aggression directed to another person by way of abuse, harassment, and malevolent behavior. It is defined as deliberately and systematically putting a persistent pressure on an individual, a group, or a worker in order to isolate that person which generally occurs in workplaces.[1,2,3,4,5,6] In the late 1980s, a German psychologist Heinz Leymann was the first to define psychological abuse taking place in workplaces.[3]

We can say that family physicians have an important place in healthcare services in Turkey without a second thought. Along with the inclusion of the family physician system as part of the transformation of the public healthcare system, many family physicians have become both physician and administrator responsible for the maintenance of coordination in their workplaces within their service area.

In association with these new responsibilities of family physicians in our country, we aimed to evaluate the mobbing knowledge and behavior of family physicians.

In this regard, we conducted a survey study by reaching every family physician working in the central district of the Malatya province. In light of the responses to the questionnaire, we made some deductions and presented the points that we deemed valuable in the solution of the problem.

Material and Method

Our study sample consisted of a total of 130 family physicians working in the central district of Malatya, a province of Turkey, in November 2012. A total of 120 physicians (92.3%) supported our study by participating in the study, whereas 10 (7.7%) were excluded from the study since they did not complete our questionnaire.

Our study questionnaire consisted of 15 items. The first 4 items were about the demographic characteristics of the participants (age, gender, marital status, working years), whereas the remaining ones were about their knowledge of mobbing, exposure to mobbing, perpetration of mobbing, and behaviors related to mobbing. The responses to this questionnaire were analyzed by SPSS 15.0 package program using Pearson's chi-squared, Yates corrected chi-squared, and Fisher's exact tests. $P<0.05$ was recognized as statistically significant.

We employed multivariate logistic regression analysis to determine the parameters (age, gender, marital status, administrative responsibility, knowing the meaning of mobbing, describing mobbing as a malevolent behavior, working years) that may be associated with the act of mobbing perpetrated by the family physicians. The goodness of fit for the estimated multivariate logistic regression model was measured with Hosmer and Lemeshow test.

Results

Our study population consisted of a total of 120 family physicians working in the central district of Malatya province in November 2012. Ten physicians who did not agree to participate in the study were excluded.

There was no significant difference between male and female genders in terms of exposure to mobbing (P=0.87).

Furthermore, there was no significant difference in exposure to mobbing with regard to marital status among family physicians (P=0.82 Yates corrected chi-squared test). However, there was a significant difference between the age groups in terms of exposure to mobbing (P=0.015 Pearson's chi-squared test).

There were significant differences between the work year groups relative to exposure to mobbing (P=0.001).

We found no difference between family physicians knowing and not knowing the definition of mobbing in terms of perpetration of mobbing (P=1).

There was a significant difference between family physicians with and without administrative responsibility in terms of perpetration of mobbing (P=0.018).

Among family physicians knowing the definition of mobbing, there was a significant difference

Table 1. Exposure to mobbing relative to gender difference

	Exposure to mobbing					
	Yes		No		Total	
Gender	**n**	**%**	**n**	**%**	**n**	**%**
Male	33	44.6	41	55.4	74	100.0
Female	19	41.3	27	58.7	46	100.0
Total	**52**	**43.3**	**68**	**56.7**	**120**	**100.0**

n: number, %: percentage, P=0.87, Yates corrected chi-squared test.

Table 2. Exposure to mobbing relative to working years among family physicians

	Exposure to mobbing					
	Yes		No		Total	
Working years	**n**	**%**	**n**	**%**	**n**	**%**
0-5	2	11.1	16	88.9	18	100.0
6-10	14	40.0	21	60.0	35	100.0
11-20	23	46.0	27	54.0	50	100.0
≥21	13	76.5	4	23.5	17	100.0
Total	**52**	**43.3**	**68**	**56.7**	**120**	**100.0**

n: number, %: percentage , P=0.001, Pearson's chi-squared test.

between those with and without an administrative responsibility with regard to perpetration of mobbing (P=0.017, Fisher's exact test).

No significant difference was found between family physicians describing and not describing mobbing as a malevolent behavior in terms of perpetration of mobbing (P=1, Fisher's exact test). Furthermore, among the family physicians describing mobbing as a malevolent behavior, there was a significant difference between those with and without an administrative responsibility in terms of perpetration of mobbing (P=0.019, Fisher's exact test).

There was a significant difference between the family physicians perpetrating and not perpetrating mobbing in terms of their behavior to the mobbing victim when they regarded themselves involved a mobbing (P=0.015, Pearson's chi-squared test).

Among the family physicians knowing the definition of mobbing, there was a significant difference between those perperating and not perpetrating mobbing in terms of their behavior to mobbing victim when they regarded themselves involved in a mobbing (P=0.016 Pearson's chi-squared test).

Among the family physicians who described mobbing as a malevolent behavior, when they regarded themselves involved in a mobbing, there was a significant difference between those perpetrating and not perpetrating mobbing in terms of their behavior to the mobbing victim (P=0.015, Pearson's chi-square test).

While 36.5% of our study population reported filing a complaint to the related superior authority in order to overcome the psychological abuse, 7.7% reported declaring their complaint through the Internet, 36.5% reported getting into a fight with the perpetrator of the mobbing, and 19.2% reported continuing to be a victim of mobbing. There was a significant difference between those who had suffered mobbing and those who had not with regard to their behavior toward dealing with mobbing when faced with one (P=0.0, Pearson's chi-square test).

Thirteen (10.8%) of the family physicians reported a trust in the privacy of procedures carried out about mobbing, whereas 107 (89.2%) reported a distrust. There was no significant difference between those trusting and not trusting the privacy of mobbing-related procedures with regard to reactions to mobbing when faced with one (P=0.64, Pearson's chi-square test).

The percentage of family physicians aware of the recent official circular regarding the "Prevention of Mobbing in Workplaces" (No, 27879; Date, 19.03.2011) was 13.3% (n=16), whereas 86.7% (n=104) did not know the circular.

Among our family physicians, 12.5% (n=15) knew the assistance and support given by psychologists via "Call 170" phone service provided by the "Communication Service of Labor and Social Security Center", whereas 87.5% (n=105) did not know about this service.

Table 3. Mobbing perpetrated by family physicians knowing or not knowing the definition of mobbing

	Ever perpetrated mobbing?					
	Yes		**No**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**
Those who know the definition of mobbing	7	6.0	109	94.0	116	100.0
Those who don't know the definition of mobb.	0	0.0	4	100.0	4	100.0
Total	**7**	**5.8**	**113**	**94.2**	**120**	**100.0**

n: number, %: percentage, P=1, Fisher's exact test.

Table 4. Importance of having an administrative responsibility in mobbing

	Ever applied mobbing?					
	Yes		**No**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**
With administrative responsibility	6	12.2	43	87.8	49	100.0
Without administrative responsibility	1	1.4	70	98.6	71	100.0
Total	**7**	**5.8**	**113**	**94.2**	**120**	**100.0**

n: number, %: percentage, P=0.018 Fisher's exact test.

The most significant factors motivating the perpetration of mobbing was having an administrative responsibility (OR=16.4; 95% CI=1.24-216.5) and working years (OR=6; 95% CI=1.21-29.3). The goodness of fit for the estimated multivariate logistic regression model was measured with Hosmer and Lemeshow test. We concluded that the model was in good compliance with the data (chi-square=2.43, df=7, P=0.93).

Conclusion and Discussion

Yildirim and Yildirim did not find a significant difference between male and female academicians working in the field of healthcare who suffered a psychological abuse.[2] In the present study, there was no difference between male/female or married/single family physicians with regard to exposure to mobbing.

Yildirim and Yildirim found that among healthcare academicians with a history of psychological abuse, 66% first preferred to have a talk with the perpetrator face to face and 51% filed a complaint to the perpetrator's superiors subsequent to this talk.[2] In our study, 36.5% of the family physicians who suffered a psychological abuse filed a complaint to the related authorities, 7.7% informed the related persons via the internet, 36.5% had a row with the mobber, and 19.2% put up with the mobbing.

In our study, mobbing rate was observed to soar with older age (71.4% for people aged 46 years and older) and longer working years (76.5% for people aged 21 years and above) due to increasing exposure to mobbing.

There was no difference in perpetration of psychological abuse between the family physicians knowing and not knowing the definition of mobbing, which suggests that a malevolent behavior may also be exhibited while being aware of its wrongful nature. Therefore, one may think that only knowledge is not effective in prevention of mobbing. Furthermore, there was no difference between family physicians describing and not describing mobbing as a malevolent behavior with regard to perpetration of mobbing.

Quine studied junior doctors and found that 37% suffered psychological abuse (2002). Yildirim and Yildirim focused on healthcare academicians and found the related exposure rate as 90% (2010).[2] Aksu and Akyol found that the rate of exposure to emotional abuse among intensive care nurses was 79% (2011).[7] Sahin et al. conducted a study among male physicians completing their military service in 2012 and observed that 87.7% of the physicians suffered mobbing.[8] In the present study, 43.3% of the family physicians participated in our study were found to have suffered mobbing.

It can be said that mobbing is quite common among healthcare workers in our country.[6] The reasons behind this are inadequate medical resources in hospitals, high number of patients, insufficient pay, bureaucratic hurdles, discrimination in academic promotions, intensive work conditions, and being close to the administration (Cobanoglu et al, 2005).[3]

In 2010, Efe and Ayvaz performed a study among the nurses at a hospital in Turkey wherein 19.4% of the nurses reported that they suffered mobbing from the physicians.[6] Yildirim and Yildirim investigated mobbing among healthcare academicians and found that their administrators were the mobbers in most of the cases.[2] In the present study, we found a significant difference between family physicians with and without an administrative responsibility in terms of exhibiting mobbing behavior. The number of family physicians who perpetrated mobbing was 6 (12.2%) in those with an administrative responsibility and 1 (1.4%) in those without an administrative responsibility. Having an administrative reponsibility appears to increase the rate of mobbing.

In the present study, the most important factors influencing the act of mobbing were having an administrative responsibility (OR=16.4; 95% CI=1.24-216.5) and working years (OR=6; 95% CI=1.21-29.3). According to our analysis, the most significant component that lays the background for mobbing is having an administrative responsibility. Mobbing rate among family physicians was found to be 16-fold higher in those with an administrative responsibility and 6-fold higher with 1 unit increase in working years.

Among family physicians who exhibited an act of mobbing or not, there was a significant difference relative to their behavior to the mobbing victim when they thought they exhibited an act of mobbing. There were differences with regard to experiencing anxiety, silencing the mobbing victim, continuing mobbing all the same, and quitting mo-

bbing due to feeling shame. The number of family physicians who did not exhibit an act of mobbing but stated that they would feel ashamed and quit mobbing if they ever perpetrate such a malevolent behavior, was the highest with 106 people (93.8%).

While mobbing is deemed as a shameful act, family physicians may suffer from it all the same.[9] In order to prevent psychological abuse that can not be prevented by just having knowledge about it and maintain a peaceful work environment, we believe that people should know their boundaries or these boundaries should be always controlled for overstepping by administrators that do not exhibit or involve in an act of mobbing.[10]

While only 16 (13.3%) family physicians knew about the circular titled as "Prevention of Mobbing in Workplaces", only 15 (12.5%) family physicians were aware of the "Call 170" phone line powered by the "Communication Service of Labor and Social Security Center" via psychologists working as part of a struggle against psychological abuse. "Call 170" phone line can also be used by family physicians for prevention of mobbing and maintenance of a peaceful workplace.[11]

References

1. *İzmir G, Fazlioğlu A. İşyerinde Psikolojik Taciz (mobbing) ve Çözüm Önerileri Komisyon Raporu. Kadin Erkek Firsat Eşitliği Komisyonu Yayinlari. No: 6. TBMM Basimevi, Ankara, 2010; S: 3-81.*

2. *Yildirim D, Yildirim A. Sağlik Alaninda Çalişan Akademisyenlerin Karşilaştiklari Psikolojik Şiddet Davranişlari ve Bu Davranişlarin Etkileri. Turkiye Klinikleri J Med Sci 2010; 30(2): 559-70*

3. *Dikmetaş E, Top M, Ergin G. Asistan Hekimlerin Tükenmişlik ve Mobbing Düzeylerinin İncelenmesi. Türk Psikiyatri Dergisi. 2011; 22(3): 137-49.*

4. *Pranjic N, Males-Bilic L, Beganlic A, et al. Mobbing, Stress, and Work Ability Index among Physicians in Bosnia and Herzegovina: Survey Study. Croat Med J.2006; 47: 750-8.*

5. *Kivimakia M, Elovainiob M, Vahterac J. Workplace bullying and sickness absence in hospital staff. Occup Environ Med 2000; 57: 656-660 doi:10.1136/oem.57.10.656*

6. *Efe SY, Ayaz S. Mobbing Nurses in the Workplace in Turkey. International Nursing Review. 2010; 57: 328-334.*

7. *Aksu T, Akyol A. Yoğun Bakim Hemşirelerinin Duygusal Tacize Maruz Kalma Durumlarinin İncelenmesi. Turkiye Klinikleri J Nurs 2011; 3(1): 29-37.*

8. *Sahin B, Cetin M, Cimen M, Yildiran N. Assessment of Turkish junior male physicians' exposure to mobbing behavior. Croat Med J. 2012 August; 53(4): 357–366. doi: 10.3325/cmj.2012.53.357*

9. *Gül H, Alçalar N, Kayi I ve ark. İş Yaşaminda Psikolojik Yildirma (Mobbing) ve Etkileri: Özel Sermayeli Bankalarda Çalişanlar Arasinda Kesitsel Bir Araştirma. Turkiye Klinikleri J Med Sci 2010; 30(6): 1917-27*

10. *Quine L.Workplace bullying in NHS community trust: staff questionnaire survey. BMJ. 1999 January 23; 318(7178): 228–232.*

11. *Genelge. "İşyerlerinde Psikolojik Tacizin (Mobbing) Önlenmesi". Resmi Gazete. 27879. 19 Mart 2011.*

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Assessment scale for psychosocial symptoms in hospitalized children scale development, reliability and validity study

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Abstract

Aims: The aims of the study were to develop and test the reliability and validity of a scale to assessment psychosocial symptoms in hospitalized children ages 6-12.

Methods: The study sample consisted of 350 children who were inpatients at the Gaziantep Pediatric Hospital. Data were collected using a Personal Information Form containgin socio-demographic information on the children and the Assessment Scale for Psychosocial Symptoms in Hospitalized Children developed by the investigator.

Findings: An item pool generated through surveying the literature was sent to experts in the field for content validity, a 3-point 57 item Likert scale was prepared. Following item analysis, 33 items with reliability coefficients smaller than .40 were removed from the scale; the remaining 24 items were grouped under five factors after confirmatory factor analysis. The Cronbach Alpha reliability coefficient of the scale was found as reliable (.90) and statistically significant ($p<.001$).

Results: The resulting "Assessment Scale for Psychosocial Symptoms in Hospitalized Children" is a reliable and valid tool.

Key words: Physically ill children, hospitalization, assessment of psychosocial problems, scale development.

Introduction

Being sick and in the hospital is a fear and anxiety invoking event that affects a child's life in various ways.[1,2,3] It is known that illness is trauma for children of all ages; and that being hospitalized has various negative effects on a child.[4,5,6,7] Children who are hospitalized experience fear of medical interventions in an alien environment with people they do not know, while having various levels of psychosocial impacts of physical damage, surgery and anxiety of being separated from their families.[5,8,9]

Children who are hospitalized have increased levels of feelings of attachment leading to separation anxiety and affection withdrawal, they also exhibit regressive behaviour such as thumb-sucking and speech impairment.[8,10] Children who are inpatients due to physical illness also display psychosocial problems such as intraversion, unhappiness, sadness, crying, detachment, desparation, weakness, guilt and feelings of worthlessness, sleep and eating disorders, irritability, restlessness.[7,11,12,13,14] These problems prolong the child's stay at the hospital and result in deterioration in the quality of life.[15,16]

Pschosocial problems are known to be ignored in the health care system despite their effect on the patient's assessment and treatment as well as their impact on changing the progression of the illness.[17,18] Whereas, these problems have to be evaluated with a wholistic approach. For this purpose, data collection instruments effective in the assessment of psychosocial symptoms are very much needed.[6,19,20,21] Diagnostic tools that are used for multiple purposes including assessment, screening, measuring impact or change are useful in helping with the correct assessment, managing the appropriate care, defining the symptoms correctly and identifying co-existing situations.[22] Despite being reliable and valid screening tools, these instruments are often inadequate in helping health professionals assess the pschological status of the hospitalized child or diagnosing psychosocial symptoms that develop in the child related to hospitalization.[23,24] For these reasons it is deemed useful to develop an instrument that can be used for hospitalized children.

The aim of this study is the development, validation and reliability of Assessment Scale for

Psychosocial Symptoms in Hospitalized Children. The instrument developed is aimed to be used by health professionals in the assessment of early stage psychological symptoms, reduce hospital stay, enhance adaptation to illness and treatment and increase quality of life in pediatric inpatient populations.

Method

This study is a methodological study implemented in the General Clinics of Gaziantep Pediatric Hospital.

Study Sample

The study sample consisted of 350 inpatient children at the Gaziantep Pediatric Hospital (The Republic of Turkey Gaziantep Provincial Health Directorate Number. B104ISM4270009-273/25493). Study inclusion criteria were: children aged 6-12 who were diagnosed with an acute or chronic disease but no psychiatric diagnoses, hospitalized for at least three days, interested in participating in the study. It is generally advised to include 5-10 subjects per scale item when determining sample size in scale development studies.[25,26] In this current instrument of 57 items, we attained more than six-fold the number patients for each item.

Data Collection Instruments

The instruments used in data collection were the Personal Information Form, assessing identifying information of the patients, and the Assessment (Draft) Scale for Psychosocial Symptoms in Hospitalized Children which was compiled by the investigators.

Personal Information Form: The Personal Information Form was prepared by the investigator has two sections. The first section contains 19 questions regarding sociodemographic information about the child and the parents such as sex, age, edutation, insurance coverage, income and place of residence. The second section contains six questions aiming to collect data about the child's illness ie diagnosis, duration, the experience of hospitalization and medical history.

The Assessment (Draft) Scale for Psychosocial Symptoms in Hospitalized Children: This instrument was developed to diagnose psychosocial symptoms in 6-12 year children who are inpatients in a hospital. The 57 scale items are in 3 point Likert scale items and scored between 0,1 or 2 for. 'never', 'sometimes', 'frequently' consecutively. The highest possible score to be obtained from the scale is '114' and the lowest is '0'. Higher scores indicate psychosocial problems in the hospitalized child.

The process

Procedures commonly followed in scale development are: searching the literature, consulting with experts in the field, adopting a commonly accepted view, adaptation of a tested instrument, collecting information from a heterogenous sample representing the population.[27] In this study aiming to develop Assessment Scale for Psychosocial Symptoms in Hospitalized Children, the first step was to determine the psychosocial symptoms in hospitalized children through a comprehensive literature search.[7,8,13,14,24,28,29,30,31,32,33,34,35]

Testing the content validity of a scale usually entails expert opinions on the subject as well as the utilization of existing theoretical and empirical studies.[27] In the current study, expert opinions were evaluated for content validity. In the process of assessing Turkish Language validity and the content validity of the draft items, the pool of items prepared were presented to 9 experts and their evaluation of the compatibility of the items with the aims, clarity and level of importance were obtained.

It is strongly recommended to conduct a pilot study to test the applicability of the data collection instrument in the field, as there is 'no turning back' if certain scale items are not clear or information retreived is found to be incomplete after data collection is completed.[36] The draft instrument must therefore be tested in a sample of 10-20 individuals who have similar characteristics with but are not included in the study sample.[27,37] In the current study, the applicability of the instrument was also tested in a small group of 10 individuals who met the study inclusion criteria but were not included in the study sample. The final version of the scale before launching the study was based on the results of this pilot application.

Written institutional permission was sought from the hospital where the study was planned. All study subjects were given information regarding the aims and importance of the study for obtaining informed oral consent.

The scale was filled-out by the investigator through direct observation of the hospitalized children. The study sample of 350 children in the study group were included in the evaluation following the third day of their hospitalization.

Data Analysis

Data were analyzed using SPSS-18 (Statistical Package for the Social Sciences). Statistical methods used in the analysis of data obtained in the study (Table 1).

Findings

The findings of the current study are presented in the following two sections:

1. Findings related to the descriptive analysis of the subjects

The study participants are children 34% of which are girls, 56.6% are boys; 33.3% of the children are of ages 6-7, 29.7 % are ages 8-9 and 37.2% are ages 10-12. Other demographic characteristics of the children are as follows: 86.3% are elementary school students, 86.3% have social security, while 54.0% are of lower income families and 47.1% live in a provincial center.

The childrens' mothers are mostly elementary school graduates (63.4%) and only 2% are employed; 98.2% of the fathers are alive, 64.8% are elementary school graduates, 93% are employed of which 62.1% are self-employed. The majority of the children have nuclear families (85.4%), 52.6% have 1-2 siblings and 27.1% are first-borns. 68% of the children are hospitalized with acute conditions and 32% have had a chronic disease for 1-12 (5.58 ± 2.95) years. 71.1% of the children have had an illness for 3-7 days, 51.4% have been in the hospital for 4-6 days while 62.6 % of the children have been hospitalized two or more times and 31.1% of them get sick frequently.

2. Findings related to scale development Reliability and validity of the scale

Content Validity: Two expert opinions were sought for Turkish Language evaluation and comprehensibility of the items of Assessment (Draft) Scale for Psychosocial Symptoms in Hospitalized Children. Some revisions were made following expert recommendations. Within the context of content validity of the draft items, the item pool was presented to 7 expert views whereby the items were assessed for compatibility with the aims, clarity and order of importance.

Evaluation of expert views were processed via the Content Validity Index (CVI) developed by Waltz and Bausell (1981) (Grant and Davis, 1997) where the expert raters were asked to score each item between '1' and '4'. In this process where the items were asked to be evaluated for clarity where responses are rated as "not relevant", "somewhat relevant", "relevant", "completely relevant" and scored from 1 to 4 consecutively. Calculated expert opinion ratings were above 3 for each item. CVI calculations require the removal of an item from the instrument if the acceptable mean score of the item is below 2.[38] When ratings for each item over 4 were summed and divided by the number of raters, the scores were 2.42-3.60. Therefore, none of the scale items were eliminated.

One of the methods used for content validity is the Davis technique. In the Davis technique, expert opinions are evaluated as a) content valid b) minor revision c) major revision d) content invalid. When using this technique, the number of raters selecting options a and b are divided by the total number of ratersto yield the content validity index (CVI) for each item. The index is not compared with a statistical criterion, but a threshold of 0.80 is utilized instead.[39] In our study we revised 4 items with a CVI of less than 0.80. The scale was finalized in its current version with 57

Table 1. Statistical method

Variables	Statistical Method
- Selected information regarding the children	Number, percentage, means, standard deviations
- Content validity	Average
- Item Analysis	Pearson moments correlation analysis
- Construct Validity	Factor analysis
- Internal consistency of the scale and its subdimensions	Cronbach's Alpha reliability coefficient

Table 1. Item-Total Score correlations of the draft scale

Items	Item –Total Score Correlation Coefficient	
	r_s	p
1.Persistently asks questions about his/her illness	**.08**	**.163**
2.Says he/she will not recover	**.15**	**.006**
3.Does not want to be hospitalized; asks to be taken home	.48	.0001
4.Wants no treatment, overreacts to any attempts at treatment	.56	.0001
5.Looks sad	.46	.0001
6.Looks unhappy	.46	.0001
7.Is uninterested in his/her environment	.57	.0001
8. Seems uneasy	.52	.0001
9. Very restless, cannot stand still	**.05**	**.380**
10.Seems irritable and angry	**.25**	**.0001**
11. Finds difficulty concentrating on any particular topic	.45	.0001
12.Wants to spend more time with his/her mother than used to	.47	.0001
13. Seems listless and tired	.41	.0001
14. Acts younger than his/her age	**.21**	**.0001**
15. Has difficulty sleeping	**.04**	**.427**
16. Constantly wants to sleep	**.20**	**.0001**
17. Seems afraid and anxious	.63	.0001
18. Often complains about pain	**.18**	**.001**
19.Claims that life is meaningless	**.11**	**.039**
20.Says he/she wants to die	**.07**	**.213**
21.Does not want to part with his/her parents; is very dependent on them	.56	.0001
22.Cries a lot	.57	.0001
23.Damages furniture, throws them around	.40	.0001
24.Has no appetite, eats less than the necessary daily amount	**.29**	**.0001**
25. Cannot get along with other children, argues with them	**.36**	**.0001**
26. Does not want to talk to anyone	.62	.0001
27. Claims no one loves him/her	**.18**	**.001**
28. Is afraid of physical harm and runs away	.63	.0001
29. Shows no interest in personal grooming, eg. does not brush his/her hair when s/he can, does not look in the mirror, etc	.40	.0001
30. Tells lies	**.25**	**.0001**
31. Bites his/her nails	**.19**	**.0001**
32.Sucks his/her thumb (or fingers)	**.23**	**.0001**
33.Has nightmares	**.12**	**.031**
34.Blames self; thinks he/she fell ill due to some wrongdoing on his/her behalf (such as not listening to Mum or treating a sibling badly)	**.03**	**.581**
35.Touches genitalia too often	**.28**	**.0001**
36. Yells and screams unnecessarily	**.23**	**.0001**
37.Seems retiring and bashful	**.38**	**.0001**
38.Stares blankly for long periods	**.28**	**.0001**
39. His/her emotions fluctuate frequently	**.30**	**.0001**
40. Swears and makes obscene comments	**.25**	**.0001**
41. Has temper tantrums. Gets angry easily	**.32**	**.0001**
42.Wets himself/herself during the day	**.01**	**.810**
43.Wets himself/herself at night	**.31**	**.0001**

44.Does not communicate with nurses and other health workers	.62	.0001
45.Refuses to sleep alone	.40	.0001
46. Does not want to share toys or personal items.	.43	.0001
47. Averts his/her eyes when talking.	.54	.0001
48. Bites and hurts the people around him/her when angry..	**.30**	**.0001**
49. Is unable to express emotions easily.	.56	.0001
50. Lays in bed all day.	**.31**	**.0001**
51. Complains about being lonely	**-.08**	**.160**
52. Tries to attract attention	**.01**	**.930**
53. Gets argumentative	.41	.0001
54. Says he/she dislikes him/herself	**.07**	**.228**
55. Refuses treatment	.59	.0001
56. Blames his/her sickness on others	**-.01**	**.912**
57. Does not listen to what he/she is told.	.44	.0001

Table 2. Explanatory factor analysis results for the assessment scale for psychosocial symptoms in hospitalized children

Scale Items	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
4. Wants no treatment, overreacts to any attempts at treatment	.76				
22. Cries a lot	.73				
17. Seems afraid and anxious	.66				
28. Is afraid of physical harm and runs away	.65				
3. Does not want to be hospitalized; asks to be taken home	.57				
55. Refuses treatment.	.55				
8. Seems uneasy.	.49				
5. Looks sad.		.86			
6. Looks unhappy		.86			
13. Seems listless and tired.		.74			
7. Is uninterested in his/her environment		.66			
29. Shows no interest in personal grooming, eg. does not brush his/her hair when s/he can, does not look in the mirror, etc		.44			
49. Is unable to express emotions easily.			.79		
47. Averts his/her eyes when talking			.76		
44. Does not communicate with nurses and other health workers			.76		
26. Does not want to talk to anyone			.56		
23. Damages furniture, throws them around				.72	
53. Gets argumentative.				.67	
57. Does not listen to what he/she is told..				.66	
46. Does not want to share toys or personal items.				.63	
11. Finds difficulty concentrating on any particular topic				.54	
21. Does not want to part with his/her parents; is very dependent on them					.79
12. Wants to spend more time with his/her mother than used to					.78
45. Refuses to sleep alone					.53
Eigenvalue	**3.45**	**3.32**	**3.01**	**2.83**	**2.02**
Variance explained by the items (%)	**14.36**	**13.83**	**12.53**	**11.80**	**8.41**
Total variance explained (%)	**60.94**				

items following the evaluation of expert opinions. The instrument was pilot-tested for applicability in a small sample of 10 subjects who were similar to but not part of the study sample. The comprehensibility of the questions in the data collection form and the expressions in the scale were thus assessed. Expressions that were not clear to the subjects were revised following the pilot test.

Item analysis

Item-Total Score correlations of the draft scale: Item-total score correlations of the 57 items for the reliability testing of the scale are shown in Table 1. The reliability correlation coefficients of the 24 items are between r_s= .40 and.6, positive in direction and statistically significant (p<.001). Coefficients for the remaining 10 items are not statistically significant (p>.05, r: -.08 - .08), while 23 items are statistically significant (p<.05, r: .11 -.38), a total of 33 items have reliability coefficients under .40 (Table 1). Following the item analysis for the reliability testing of the draft scale, 33 items with item-total score correlation coefficients under .40 were removed form the scale.

Construct Validity/Factor Analysis: In order to determine the construct validity of the scale, an explanatory factor analysis was performed (Table 2). Principal components analysis and varimax rotation methods were used to inspect the factor structure.

The explanatory factor analysis of the remaining 24 items following the item analysis yielded a Kaiser-Meyer-Olkin (KMO) constant of .89 and a statistically significant Bartlett test result ($\chi^2 = 3824.492;\ df: 276\ p < .0001$).

In the explanatory factor analysis, scale items were grouped in five factors that had eigenvalues above '1' (2.02-3.45). Variance explained by the factors was between 8.31% and 14.36% while the five factors contributed to 60.94% of the variance. (Table 2). The first factor was titled 'anxiety', the second 'hopelessness', the third 'communication problems' and the fourth and the fifth factors were 'anger and agression' and 'regression' consecutively.

A confirmatory factor analysis (CFA) was performed to confirm the compatibility of the five factors that were identified after the explanatory factor analysis. RMSEA and GFI fit indexes of the scale were were not satisfactory, although close, in the initial confirmatory factor analysis (Table 3). The fit indexes calculated following modification of error variances between the 3rd-4th and 55th-57th items were satisfactory in the second confirmatory factor analysis. CFA fit values are presented in Table 3. Factors loadings of all items in their related dimensions were between .45 and .87.

Item-Total correlation analysis of sub-dimensions: Item-total correlation analysis was repeated and item- sub-dimension analyses were performed on the 24-item final version of the scale with items retained after the preliminary item analysis and factor analysis. (Table 4).

All items had positive and statistically significant item-total reliability coefficients of r: .30 - .69. (p<.001, Table 4). Three items in the fourth factor (items 23, 46 and 53) had reliability coefficients of .30 - .39. These items, despite having reliability coeffients smaller than .40, were retained in the scale as their reliability coefficients of individual dimensional relationship were high.

Reliability coefficients between the five sub-dimensions of the scale and the total score were r: .58 - .80 in the first factor, r: .54 -.83 in the second, r:

Table 3. Confirmatory factor analysis fit values for the assessment scale for psychosocial symptoms in hospitalized children

DFA Fit values	Preliminary CFA results	CFA results following modification
Chi-square / p value	822.0/p = 0.00 (p<.05)	757.47 /p = 0.00 (p<.05)
Degrees of Freedom	242	240
Chi-square: Degrees of Freedom	822.0:242= 3.40	757.47:240= 3.16
RMSEA / p	0.083 (p< .05)	0.79 (p< .05)
SRMR	0.077	0.076
CFI	0.94	0.95
NNFI	0.93	0.94
GFI	0.84	0.85
AGFI	0.80	0.81

.74 - .90 in the third, r: .49 - .76 in the fourth, and r: .64 -.88 in the fifth dimension; all positive in direction and statistically significant (p<.001, Table 4).

The correlation of the scale sub-dimensions with the total score yielded positive and statistically significant r coefficients between .58-.88 (p<.001, Table 5)

Table 4. Second-Stage Item-Total correlations of the scale and its sub-dimensions

Scale Items	Item-Total correlations		Item - Sub-Dimension correlations	
	r_s	p	r_s	p
Anxiety				
4. Wants no treatment, overreacts to any attempts at treatment	.62	.0001	.80	.0001
22. Cries a lot	.62	.0001	.74	.0001
17. Seems afraid and anxious	.69	.0001	.74	.0001
28. Is afraid of physical harm and runs away	.64	.0001	68	.0001
3. Does not want to be hospitalized; asks to be taken home	.53	.0001	.71	.0001
55. Refuses treatment.	.59	.0001	.63	.0001
8. Seems uneasy	.54	.0001	.58	.0001
Hopelessness				
5. Looks sad	.53	.0001	.80	.0001
6. Looks unhappy	.50	.0001	.83	.0001
13. Seems listless and tired	.50	.0001	.79	.0001
7. Is uninterested in his/her environment	.59	.0001	.64	.0001
29. Shows no interest in personal grooming, eg. does not brush his/her hair when s/he can, does not look in the mirror, etc	.54	.0001	.54	.0001
Communication Difficulty				
49. Is unable to express emotions easily.	.63	.0001	.89	.0001
47. Averts his/her eyes when talking.	.58	.0001	.75	.0001
44. Does not communicate with nurses and other health workers	.69	.0001	.90	.0001
26. Does not want to talk to anyone	.67	.0001	.74	.0001
Anger and agression				
23. Damages furniture, throws them around	.34	.0001	.49	.0001
53. Gets argumentative	.30	.0001	.76	.0001
57. Does not listen to what he/she is told	.40	.0001	.62	.0001
46. Does not want to share toys or personal items.	.39	.0001	.54	.0001
11. Finds difficulty concentrating on any particular topic	.41	.0001	.63	.0001
Regressive behaviour				
21. Does not want to part with his/her parents; is very dependent on them	.58	.0001	.88	.0001
12. Wants to spend more time with his/her mother than used to	.50	.0001	.86	.0001
45. Refuses to sleep alone	.43	.0001	.64	.0001

Table 5. Correlations of the sub-dimension scores and the total score of the Assessment Scale for Psychosocial Symptoms in Hospitalized Children

Sub-dimensions	Sub-dimension-Total score correlation coefficients	
	r	p
Anxiety	.88	.0001
Hopelessness	.71	.0001
Communication Difficulty	.78	.0001
Anger and agression	.58	.0001
Regressive behaviour	.63	.0001

Internal consistency and reliability coefficients: As a result of the analysis performed to test internal consistency, a reliability indicator of the scale and its sub-dimensions, the Cronbach Alpha reliability coefficient was calculated as $\propto$ = .90 for the instrument. Internal reliability of the sub-dimensions of the scale yielded reliability coefficients of .86 infactor 1 (anxiety), .84 in factor 2 (hopelessness), .83 in factor 3 (communication difficulty), .69 in factor 4 (anger and agression), and .74 in factor 5 (regression).

Discussion

This study was conducted for the development, reliability and validity of the Assessment Scale for Psychosocial Symptoms in Hospitalized Children. First, the item-total correlations of the scale draft were explored. Item analysis is used to determine how each item in the instrument contributes to the total score thus determining the relationship of the item with the whole of the instrument.[40] The larger the correlation coefficient for each item, the stronger the connection of the item with the theoretical construct measured, indicating the effectiveness and adequacy of the item in measuring the targeted behaviour. Although not definite, coefficient values acceptable in selecting items are expected to be .25 or above. [36,40] The reliability analysis of the 57 scale items showed that, item-total correlations coefficients of 24 items were positive, between .40 -.63 and statistically significant (p<.001).

Validity of scales frequently involve explanatory factor analysis, while exploration of the factors involves principal component analysis and the varimax technique.[25] In the current study, factor structure was explored using principal component analysis and the varimax rotational technique.

Factor analysis involves calculations on the correlation matrix obtained by inter-item correlations, while the significance of the correlation coefficients between items are explored with Barlett's test of sphericity. The p values should be significant.[41] The adequacy of the sample is determined by the Kaiser-Meyer-Olkin (KMO) measure.[25] For a good factor analysis, the KMO value is recommended to be above .60.[41] The exploratory factor analysis of the scale yielded a KMO coefficient of .89 and a statistically significant Bartlett test result ($\chi^2 = 3824.492;\ df: 276\ p < .0001$). The KMO value of .89 indicates sampling adequacy, the significance (p<.0001) shows that the correlation matrix is factorable.

Eigenvalues are used to decide on the number of significant factors and the calculation of the proportion of the variance each factor accounts for. The Eigenvalue is the square of the factor loads for each factor. Increases in the Eigenvalue indicates and increase in the proportion of the variance explained by the factor. It is customary to select factors with eigenvalues of 1 or more as significant factors, however, depending on the analysis results this threshold may be elevated. The recommended criteria for the factor loading/factor coefficient that explains the connection of the items to the factor is usually .40 or above.[41] Factor loadings for studies with sample sizes of 350 and above are recommended to be above .30 where factor loadings of .50 and above are considered to be strong.[42] In the current study, the calculated factor loadings for all items were above .40 (.45-.87).

In the confirmatory factor analysis, fit statistics need to be at a desired level. Fit statistics are:

Chi-square statistic for fit: For a model to be acceptable, the chi-square statistic is expected not to be statistically significant, but it is usually the opposite in practice, because this value is very sensitive to sample size. Instead, the chi-square is divided by the degrees of freedom. If the result is two or below, the model is considered a good model; if the result is five or less, the model has an acceptable level goodness of fit.[43,44] Confirmatory factor analysis results of the Assessment Scale for Psychosocial Symptoms in Hospitalized Children yielded this value as 3.16; confirming an acceptable goodness-of-fit.

Other frequently used goodness-of-fit test are Root Mean Square Error of Approximation (RMSEA), Standardized Root-mean-Square Residual (SRMR), Comparative Fit Index (CFI), Non-Normed Fit Index (NNFI), Goodness of Fit Index, (GFI), Adjusted Goodness of Fit Index (AGFI)'. RMSEA values of 0.080 or less and p values of .05 or less indicate better goodness of fit, RMSEA values of .10 or less show weaker goodness-of –fit.[43,44] In the current study, the RMSEA is statistically significant and less than .80 (.79) indicating better goodness-of-fit. SRMR values less than .10, CFI, NNFI

values equal or above 0.90 , AGFI values equal or above 0.80 show good fit.[43,44] GFI values larger than 0.85 indicate an acceptable fit.[45] The SRMR, CFI, NNFI, GFI and AGFI goodness of fit measures calculated in the current study show acceptable fit.

The statistical significance of correlation coefficients between sub-dimension scores and the total score are considered to be one of the indicators of internal consistency.[26] The correlation of sub-dimension scores and the total score are calculated as r:.58-.88 indicating a positive, statistically significant correlation ($p<.001$).

For estimating the reliability of a Likert scale, the first step is to look at the Cronbach- Alpha coefficient. This coefficient is a measure of the internal consistency of the scale items. The measure of internal consistency is prior and necessary. The most suitable way of measuring internal consistency is the calculation of the Cronbach-Alpha reliability coefficient and this coefficient must be close to 1.[27] If the coefficient alpha value is less than .40, the instrument is not reliable, .40-.59 indicates low reliability, .60-.79 is considered reliable and .80-1.00 indicates high reliability.[25] In the current study, the calcultated Cronbach-Apha coefficient for the whole scale is $\propto = .90$. Internal consistency reliability for the sub-dimensions of the scale were .86 for factor 1, .84 for factor 2, .83 for factor 3, .69 for factor 4 and .74 for factor 5.

Results

24 item, five-factor, 3-point Likert scale instrument designed to assess psychosocial symptoms in 6-12 year-old inpatient children was developed as a result of the current study. The instrument has a calculated Cronbach-Alpha coefficient of .90 and a high reliability and validity level. The use of this instrument will facilitate early assess and intervention of psychosocial symptoms in hospitalized children.

References

1. *Franck LS, Kools S, Kennedy C, Kong SKF, Chen JL, Wong TK. The symptom experience of hospitalised Chinese children and adolescents and relationship to pre-hospital factors and behaviour problems. International Journal of Nursing Studies, 2004; 41(3): 661-669.*
2. *Gultekin G, Baran G. Illness and Child, Journal of Family and Community Education, Culture and Research, 2005; 2(8): 61-68.*
3. *Morris J. When a Child Is Hospitalized. Tips and Resources for Parents, 2011; www.kc.vanderbilt.edu Retrieved on 17 June 2011.*
4. *Akay AP, Emiroglu FNI. Chronic Pediatric Illness and Hospitalization. In: Cetin, F.C., Coskun, A., Iseri, E. and et al. (Eds.), Textbook of Child and Adolescent Psychiatry. HYB Publisher, Ankara, 2008; 723-729.*
5. *Cavusoglu H. Effects of Hospitalization on Child and Family. In: Child Health Nursing, Volume 1, Ankara 2004; 51-67.*
6. *Das S, Mukherjeel A, Lodhal R, et al. Quality of Life and Psychosocial Functioning of HIV Infected Children. Indian Journal of Pediatrics, June, 2010; 77: 633-637.*
7. *Theofanidis D. Chronic Illness in Childhood: Psychosocial Adaptation and Family. Health Science Journal, 2007; April-June, 1 (2), http://www.hsj.gr/volume1/issue2/issue02_rev01.pdf Retrieved on 2 February 2011.*
8. *Baykara N. Hospitalized Children According to Age Groups. In: Child and Young in Hospital. Gazi Bookstore, Print 1, Ankara,2006; 7-57.*
9. *Salmela M, Salantera S, Aronen E. Child-Reported Hospital Fears in 4 to 6-Year-Old Children. Pediatric Nursing, September-October, 2009; 35(5): 269-303.*
10. *Tuzun U. Child Psychiatry Consultation. In: Polvan, O. (Ed.), Textbook of Child and Adolescent Psychiatry. Print 1, Nobel Medicine Bookstores, Istanbul, 2000; 206-211.*
11. *Baykara A, Guvenir T, Miral S. Effects of Illness and Hospitalization on Child. In: Eksi, A. (Ed.), I'm not Sick, Psychosocial Aspects of Child Health and Diseases. Nobel Medicine Bookstores, Istanbul, 2011; Print 2: 398-402.*
12. *Cavusoglu H. Effects of Hospitalization on Child and Family. In: Child Health Nursing, Dizgi Publisher, Ankara, 2008; Volume 1: 58-67.*
13. *Lau BWK, Tse WWC. Psychological Effects of Physical Illness and Hospitalization on the Child and the Family. J.H.K.C. Psych. 1993; 3: 9-18.*
14. *Priest H. Effective Psychological Care for Physically Ill Patients in Hospital. Nursing, Standard, 2010; 24(44): 48-56.*
15. *Legg MJ. What Is Psychosocial Care And How Can Nurses Better Provide It To Adult Oncology Patients. Australian Journal of Advanced Nursing, 2011; 28(3): 61-67.*
16. *Yeo MSM, Sawyer SM. Psychosocial Assessment for Adolescents and Young Adults with Cancer. Cancer Forum, March, 2009; 33(1): 18-22.*

17. *Rushton J, Bruckman D, Kelleher K. Primary Care Referral of Children With Psychosocial Problems. Arch Pediatr Adolesc Med, June, 2010; 156: 592-598.*

18. *Wissow LS, Larson S, Anderson J, et al. Pediatric Residents' Responses That Discourage Discussion of Psychosocial Problems in Primary Care. Pediatrics, 2005; 111(6): 1569-1577.*

19. *Horwitz SM, Leaf PJ, Leventhal JM. Identification of Psychosocial Problems in Pediatric Primary Care. Arch Pediatr Adolesc Med, July, 1998; 152: 367-371.*

20. *Jellinek M, Murphy JM. Psychosocial Problems, Screening, and the Pediatric Symptom Checklist. 1999. http://www.hope.usu.edu/uaimh/html/statemodelProac/Screening%20and%20Assessment/jellinek.pdf Retrieved on 04 December 2010.*

21. *Reijneveld SA, Vogels AGC, Hoekstra F, et al. Use of the Pediatric Symptom Checklist for the Detection of Psychosocial Problems in Preventive Child Healthcare. BMC Public Health, 2006; 6: 197.*

22. *Aydemir O. Psychiatric Measures. In: Koroglu, E., Gulec, C. and Senol, S. (Eds.), Textbook of Psychiatry. HYB Publisher, Ankara, 2007.*

23. *Duke N, Ireland M, Borowsky IW. Identifying Psychosocial Problems Among Youth: Factors Associated With Youth Agreement on a Positive Parent-Completed PSC-17. Child: Care, Health-Development, 2005; 31(5): 563–573.*

24. *Emiroglu FN, Akay AP. Chronic Illnesses, Hospitalization and Child. Medicine Journal of Dokuz Eylul University, May 2008; 22 (2): 99-105.*

25. *Akgul A. Statistical Analysis Techniques for Medical Research, SPSS Applications, Print 2, Emek Offset, Ankara, 2003.*

26. *Oner N. Examples of Psychological Tests Used in Turkey, A Reference Source. Extended Print 2, Bogazici University Publisher, Istanbul, 2006.*

27. *Tezbasaran AA. Likert Type Scale Preparation Guide. 2008; Version 3, e-book, Mersin.*

28. *Board R. School-Age Children's Perceptions of Their PICU Hospitalization. Pediatric Nursing, May-June, 2005; 31(3): 166-175.*

29. *Dalton R, Forman MA. Healthy Child, Psychosocial Problems. In: Behrman, R.E., Kliegman, R.M. and Jenson, H.B. (Eds.), Akcay, T. (Trans.), Pediatry. Nobel Medicine Bookstores, 2008; Volume 1: Istanbul, 70.*

30. *Erdogan A, Karaman MG. Identification and Management of Mental Problems in Child and Adolescent with Chronic and Fatal Illness. Anatolian Journal of Psychiatry, 2008; 9: 244-252.*

31. *Erermis HS. Child and Adolescent Against of Illness. In: Cetin, F.C., Coskun, A, Iseri, E. and et al. (Eds.), Textbook of Child and Adolescent Psychiatry. HYB Publisher, Ankara, 2008; 730-737.*

32. *LeBlanc LA, Goldsmith T, Patel DR. Behavioral Aspects of Chronic Illness in Children and Adolescents. Pediatr Clin N Am, 2003; 50: 859- 878.*

33. *Peek G, Melnyk BM. Coping Interventions for Parents of Children Newly Diagnosed with Implications for Clinical Practice and Future Research. Pediatric Nursing, November-December, 2010; 36(6): 306-313.*

34. *Peykerli G. Psychological Approach to Fatal Diseases. Medicine Journal of Cumhuriyet University, 2003; 25(4): 62-65.*

35. *Zengerle-Levy K. Nursing the Child Who is Alone in the Hospital. Pediatric Nursing, May-June, 2006; 32(3): 226-237.*

36. *Aksakoglu G. Research Methods and Analysis Methods in Health. Dokuz Eylul University, Rectorate Publisher, Izmir, 2001.*

37. *Kubilay G. Data Collection, Methods, Techniques and Tools. In: Erefe, I. (Ed.), Research in Nursing, Principles, Process and Methods. Odak Offset, Print 3, Ankara, 2004; 139-167.*

38. *Gozum S, Aksayan S. Guidelines for Cross-cultural Scale Adaptation II: Psychometric Properties and Cross-Cultural Comparison. Journal of Research and Development in Nursing, 2003; 5: 1-25.*

39. *Davis LL. Instrument Review: Getting The Most From a Panel of Experts. Applied Nursing Research, 1992; 5: 194-7.*

40. *Ozdamar K. Package Programs and Statistical Data Analysis. Print 4, Kaan Bookstore, Eskisehir, 2002.*

41. *Buyukozturk S. Factor Analysis: Basic Concepts and Using to Development Scale Educational Administration in Theory and Practice, Fall, 2002; 32: 470-483.*

42. *Kalayci S. SPSS Applied Multivariate Statistical Techniques. Print 1, Asil Publisher, Ankara, 2005.*

43. *Harrington D. Confirmatory Factor Analysis. Oxford University Press, Newyork, USA, 2009.*

44. *Simsek OF. Introduction to Structural Equation Modeling, Basic Principles and LISREL Applications. Cem web Offset, Ankara, 2007.*

45. *Schermelleh-Engel K, Moosbrugger H, Muller H. Evaluating the fit of Structural Equation Models: Tests of Significance and Descriptive Goodness-of-fit Measures. Methods of Psychological Research, 2003; 8(2): 23-74.*

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The ageism attitudes of older persons'relatives and their relations

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Abstract

Objective: This study was conducted in order to identify the ageism attitudes of the relatives who live together with an elderly person and to identify the relation of these attitudes with their demographic-socio-cultural characteristics.

Material and Methods: This descriptive study was conducted on 968 people aged between 18-65 years old living in the same house with people aged 65 and over. As data collection tools, a Personal Information Form and the Ageism Attitude Scale (AAS) were used.

Results: The mean AAS total score of the relatives of the elderly was 85.5±7.9 and their attitudes towards the elderly and ageism were positive. The total AAS scores and positive discrimination against the elderly were found to be significantly higher in men than in women ($p<0.05$). According to educational level, the scores concerning the limitations on the life of the elderly were significantly higher in those with primary or high school education ($p<0.05$). The AAS total scores of people with an extended family structure were significantly higher than those with a nuclear family ($p<0.05$). The scores concerning the limitations on the life of the elderly, negative discrimination and total AAS were found to be significantly higher in respondents who lived with a first degree relative than in those living with an elderly person who was not a first degree relative ($p<0.05$).

Conclusion: It was found that the relatives of the elderly have a positive attitude towards ageism. Being male, having an extended family structure and the elderly person being a first degree relative are factors that improve the positivity of this attitude.

Key words: Elderly, ageism, relative of an elderly person.

Introduction

As a result of economical development, an increase in educational level, sufficient and balanced diet, extension of health services and technological development, child mortality, and infant mortality in particular have decreased significantly and owing to these positive developments life expectancy has increased and the proportion of old people in the population has increased (1). A decrease in the rates of mortality and fertility, which are the main determinants of population structure, has led to the world's population getting older (2,3).

Today, an ageing population is regarded as a social and economical problem, it is not only a process that is experienced by developed countries; but also by developing countries. This demographic change is accompanied by problems in social and economical areas as well as in the area of health (2).

Turkey is one of the countries that is ageing fast. Since the 1950s, with an increase in life expectancy and a decrease in the fertility rate, the number of people in the population aged 65 and over has grown steadily. In 1955, while 3.4 % of the population was aged 65 and over, this proportion increased to 4.3 % in 1990 and 5.5 % in 2000 (3).The proportion of the elderly population has risen to 6.8 % according the findings of the 2008 Turkey Demographic and Health Survey and to 7.3 % according to Turkish Statistics Institute (4.5).

It is estimated that this rapid increase in the elderly population in both developing and developed countries and the differences between the sexes may in future lead to important problems in family and society around the world. These problems concern increasing demands health services, covering the cost of health expenses, organising and financing social security institutions and the provision of adequate services and job opportunities. Moreover, it

is also reported that elderly people may have problems with such matters as adaptating to the ageing process and social support provided by their family and close friends, having an adequate income, retirement and housing etc. (1,7-11).

The changes in the socio-cultural structure of society have given rise to some changes in the perception of old age by individuals and society and consequently ageism ensues (11-13). The term "ageism" was first used by gerontologist Robert Butler, the Director of US National Institute on Aging in 1969 (14,15). While Butler defined ageism as a term that may be put into action as discrimination against the elderly, Justas in discrimination against race and discrimination against sex, Palmore defined it as a "term that is expressed through the prejudice, attitude and behaviour towards old aged people". A number of surveys have been conducted to determine the views and attitudes of the elderly and society towards ageism (13,15-19,22,23). It is stated that the fields in which ageist attitudes are experienced the most are in working, family, social and sexual lives and in such the health care system (11,24).

In spite of the positive impacts of old age as esteem, experience etc. on the individual and his environment, for various reasons such as the necessity to cope with its adversities like illness, loss of productivity, feeling of being a burden, exclusion and the necessity to create a social, administrative and organisational background, it is increasingly important to identify the attitudes towards ageism and to direct them in a positive way. In the literature, it is stressed that it is extremely important for family members to benefit from the knowledge and life experience of older family members and to develop positive attitudes towards the elderly so as to preserve the family unity (16,22). Therefore, there is a need for studies in which the attitudes of elderly people's relatives towards ageism are identified.

This study was conducted to determine the attitudes of the relatives of elderly, people who live together with an elderly person, towards ageism and the relation of these attitudes with demographic-socio-cultural characteristics.

Material and methods

This descriptive study was conducted in the Central Hacilar Province of Kayseri city. The population of the study consisted of 975 respondents aged 18-65 years old who shared their homes with an elderly person aged 65 or over. The whole population was targeted and 968 people aged 18-65 who shared their homes with somebody aged 65 or over were reached. The rate of participation in the study was found to be 99.2 %. The required official approval was obtained and the study was approved by Erciyes University's, Ethics Committee; the written consent of the participants in the study was also obtained.

Data Collection Tool

As data collection tools, a personal information form and the Ageism Attitude Scale (AAS) were employed. The data were collected by the researcher through face to face communication in the homes of the participants between 4th March and 4th May 2011. The personal information form consists of questions aiming to determine the socio-demographic characteristics of the elderly and their relatives and to determine their experiences concerning the concept of old age and living with an older person(s).

The Ageism Attitude Scale (AAS) is a 23 item scale. The AAS was developed by Vefikuluçay in 2008 and its validity-reliability studies were conducted. As the scores received from the scale increase, the positive attitudes towards ageism increase. The scale is a Likert type scale that has the options of "I strongly disagree", "I disagree", "I am not decided", "I agree" and "I strongly agree" for each item which states certain points the elderly. In the scale, the items that display a positive attitude towards ageism were scored with a 5 if they totally agree; 4 if they agree; 3 if they are not decided; 2 if they disagree and 1 if they strongly disagree. The items concerning a negative attitude towards ageism were scored just inversely (15). The maximum score to be achieved from the scale is 115 and the minimums score is 23. In our study, the reliability of the AAS was tested using Cronbach's alpha internal consistency method and the Cronbach α value was found as 0.68.

The AAS is composed of three dimensions that cover subjects such as the limitations on the life of older people, positive ageism and negative ageism. The maximum score that the dimension of limitations on the life of older persons can attain is "45"

and the minimum score is "9". The maximum score that the dimension of positive ageism can attain is "40" and the minimum score is "8". The maximum score that the dimension of negative ageism can attain is "30" and the minimum score is "6". (16).

Statistical Analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS) for Windows 17.0 statistics package program. Test of normality was employed by the Shapiro-Wilk test. In the comparison of the two groups, for the normally distributed variables, the Independent Two Sample t-test was employed while for the abnormally distributed ones the Mann-Whitney U test was employed. In the comparison of three or more groups, for the normally distributed variables Single Direction Variance Analysis was employed, while for the abnormally distributed ones Kruskal-Wallis Analysis was employed. In the Single Direction Variance Analysis, for the homogeneously variant groups as understood from the multiple comparisons of the groups with differences the Turkey test was employed, while for the inhomogeneous variant groups the Dunn's test was employed. The level of significance was 0.05 and it was found that there was a significant difference if $p<0.05$.

Results

The study included 968 respondents aged 18-65 years old who shared their homes with individuals aged 65 and over.

Of the respondents included in the study, 39.0 % were men while 61.0 % were women. When viewed from sex differences, it was found that the total AAS scores and positive ageism scores of the men were significantly higher than those of the women ($p<0.05$).

Of the respondents living with the elderly, 33.4 % were aged 33-40 years old while 28.1 % were between 25-32 years old and there was no significant difference between the mean scores of the AAS total and sub-dimension scores of the age groups ($p>0.05$).

Of the relatives of the elderly, 3.1 % were literate, 37.6 % were primary school graduates, 22.7 % were secondary school graduates of, 23.3 % were high school graduates of and 11.9 % were university graduates. According to the educational status of the respondents, the scores of the limitation on the life of the elderly were found to be significantly higher in primary and high school graduates ($p<0.05$).

In view of the occupations of the respondents, there was not a significant difference between the mean total AAS and sub-dimension scores ($p>0.05$). In view of their marital status, 83.0 % were married while 17.0 % were single and there was no significant difference between the mean total AAS and sub-dimension scores ($p>0.05$)

Of the respondents, 47.6 % were first degree relatives of the elderly person while 52.4 % were not. The difference between the mean total AAS, positive ageism and negative ageism scores ($p>0.05$) was significantly higher in the respondents who were first degree relatives of the elderly person than in those who were not.

When the respondents' AAS score medians were analysed according to their income levels, it was observed that although the AAS score medians of the respondents with low income were lower than those with good income, the difference between the groups was not significant ($p>0.05$). Although the AAS scores of the respondents with a children (95.8 %) were higher than those of respondents with no children (4.2 %), the difference between the groups was not significant ($p>0.05$).

Although the AAS scores of the respondents who lived in a village were generally higher than those of respondents who lived in a town or city, the difference between the groups was not significant ($p>0.05$).

Table 1. Ageism Attitude Scale (AAS) Scores of the relatives of the elderly

The ageism attitude scale and its sub dimensions	X±SD	Median (Min-Max)
Limitation on the life of the elderly	36.0±3.8	36.5 (16.0-45.0)
Positive ageism	32.3±4.0	33.0 (9.0-40.0)
Negative ageism	17.2±3.6	17.0 (7.0-29.0)
The AAS total score	85.5±7.9	86.0 (56.0-109.0)

SD: Standard deviation, min-max: minumum-maximum

Table 2. The distribution of the AAS scores of the relatives of the elderly according to their socio-demographical characteristics

Variables	n (%)	Limitation on the life of the elderly X±SS	Positive Ageism X±SS	Negative Ageism X±SS	Total AAS Score X±SS
Sex					
Female	590 (61.0)	35.9±3.7	31.9±4.1	17.0±3.6	85.0±8.0
Male	378 (39.0)	36.1±3.8	32.8±3.9	17.4±3.5	86.4±8.0
p		0.461	**0.001***	0.114	**0.006***
Age Group					
18-24	129 (13.3)	35.8±3.7	32.3±4.4	17.0±3.6	85.2±8.0
25-32	272 (28.1)	36.3±3.5	32.4±3.6	17.0±3.6	85.9±7.2
33-40	323 (33.4)	35.9±3.9	31.9±4.3	17.5±3.4	85.4±8.5
41-48	164 (16.9)	36.3±3.7	32.7±3.5	16.9±3.7	86.0±7.5
49-56	61 (6.3)	35.1±4.0	32.1±4.8	17.4±3.7	84.7±8.9
57 ↑	19 (2.0)	35.0±5.2	32.5±5.1	16.8±3.7	84.4±9.2
p		0.188	0.478	0.539	0.786
Educational Status					
Illiterate	14 (1.4)	35.5±3.2	33.3±3.0	15.5±3.2	84.4±5.3
Literate	30 (3.1)	36.0±4.9	31.5±5.0	18.7±4.1	86.2±10.0
Primary School	364 (37.6)	35.6±3.7	32.3±3.9	17.0±3.6	85.1±7.9
Secondary School	220 (22.7)	36.1±3.8	32.0±4.0	17.0±3.6	85.2±7.9
High School	225(23.3)	36.7±3.6	32.7±3.6	17.2±3.4	86.8±7.3
University	115(11.9)	35.6±3.7	31.9±5.0	17.6±3.6	85.1±8.8
p		**0.019***	0.339	0.071	0.259
Occupation					
Housewife	455 (47.0)	35.9± 3.7	32.0±4.0	17.1±3.6	85.1±8.0
Civil Servant	187 (19.3)	36.4 ± 3.7	32.3±4.6	17.3±3.5	86.0±8.4
Worker	192(19.8)	36.1 ± 3.7	32.5±3.6	17.4±3.5	86.0±7.4
Other	134 (13.9)	35.6 ± 4.3	32.8±3.9	17.2±3.6	85.7±7.9
p		0.404	0.135	0.775	0.382
Family Type					
Nuclear family	109 (11)	35.6±4.1	31.7±4.3	16.8±3.6	84.1±8.5
Extended family	859 (89)	36.0±3.7	32.3±4.0	17.2±3.6	85.7±7.9
p		0.227	0.106	0.217	**0.049***
Marital Status					
Married	804 (83.0)	36.0±3.8	32.2±4.0	17.2±3.6	85.6±7.9
Single	137 (17.0)	35.8±3.6	32.4±4.2	17.1±3.6	85.4±8.1
p		0.536	0.678	0.721	0.806
Degree of relativity					
First Degree	461 (47.6)	36.0±3.8	32.6±4.0	17.4±3.5	86.0±8.0
Not first degree	507 (52.4)	36.0±3.7	32.0±4.1	16.9±3.6	85.0±7.9
p		0.760	0.034*	0.036*	**0.029***
Income level					
Good	241 (24.9)	36.0±3.8	32.6±4.4	17.4±3.9	86.1±8.2
Average	687 (71.0)	36.0±3.8	32.2±4.0	17.1±3.5	85.3±7.9
Poor	40 (4.1)	35.9±3.5	32.3±3.3	16.9±3.6	85.2±7.6
p		0.959	0.388	0.454	0.386

**p<0.05, AAS: Ageism Attitude Scale*
SD: Standard deviation

Of the respondents, 33.9 % had lived with an elderly relative for 1-4 years while 40.2 % of them had done so for 10 years or more and the difference between their AAS total scores and sub-dimension mean scores was not significant (p>0.05).

It was found that as many as 86.9 % of the elderly did not work and no health was observed in problem 62.9 % of the elderly individuals. The difference between their AAS total scores and sub-dimension mean scores concerning these variables was not significant (p>0.05).

Discussion

In this study, it was found that the old age and ageism attitudes of the respondents who live with an elderly person were positive. It is thought that these positive attitudes are the result of the fact that some inherited traditional behaviour patterns such as respect for the elderly, obeying the elderly and protecting them have an important place in Turkish culture.

In a great many studies on ageism, the opinions of children and young people were surveyed (16,26,27-30,22,23). A study was conducted by Palmore in order to identify the perception of ageism and the frequency of encountering ageism in Canada and the United States of America (USA). In the study, it was found that older people were exposed to negative ageism on such subjects as benefiting from health services, finding a job, promotion, finding a house and getting a loan. According to other results obtained from the study, it was observed that the elderly stated that society considers them as individuals who cannot hear properly, who have difficulty in understanding what they are told and who are constantly sick because of their age (31).

In a study by Cheung et al. on the attitudes of social workers and the society in Japan concerning the elderly, it was found that elderly people were regarded as responsible and conscious individuals. Furthermore, in that study it was found that social workers and society stated that it did not take the

Table 3.

Variables	n (%)	Limitation on the life of the elderly X±SD	Positive Ageism X±SD	Negative Ageism X±SD	Total AAS Score X±SD
Being a parent					
Yes	796 (95.8)	36.0±3.7	32.2±4.0	17.2±3.5	85.6±7.9
No	35 (4.2)	36.3±5.2	32.1±4.3	16.3±4.1	84.8±9.9
p		0.765	0.810	0.145	0.557
Usual place of abode					
City	188 (19.4)	35.9±3.7	31.7±4.4	17.0±3.4	84.7±8.0
Town	748(77.3)	36.0±3.8	32.4±4.0	17.2±3.6	85.7±8.0
Village	32(3.3)	36.5±2.8	33.0±3.9	16.5±3.6	86.2±6.7
p		0.684	0.103	0.444	0.316
Time lived together					
1 year↓	71(7.3)	36.4±3.7	31.4±5.3	17.2±3.8	85.2±9.2
1-4 years	328(33.9)	36.1±3.5	32.5±3.8	17.2±3.6	85.9±7.5
5-9 years	180(18.6)	35.6±4.0	32.0±4.2	17.5±3.3	85.2±8.3
10 years ↑	389(40.2)	36.0±3.9	32.3±3.8	17.0±3.6	85.4±7.9
p		0.408	0.147	0.657	0.743
Working capacity of the elderly					
Available	127(13.1)	35.8±3.9	31.8±4.1	17.4±3.3	85.1±7.9
Non-available	841 (86.9)	36.0±3.8	32.3±4.0	17.1±3.6	85.6±7.9
p		0.516	0.196	0.414	0.546
Health problems of the elderly					
Available	608(62.9)	36.1±3.8	32.4±4.1	17.2±3.6	85.8±8.0
Non-available	360 (37.1)	35.8±3.7	32.0±4.0	17.2±3.5	85.1±7.7
p		0.247	0.121	0.974	0.183

elderly any longer to learn a new thing than it did for young people, that their reduced senses were not connected to their age and that older people should earn enough to live (17).

It is noticeable today that those who display discriminatory behaviours against the elderly are mostly young people. There are several studies indicating that university students have negative attitudes towards the elderly (25,26,32,34-38). In a study by Moyle on the attitudes and perceptions of nursing students towards older persons, it was found that nursing students had negative attitudes towards the elderly and they perceived them as mostly oversensitive, weak and sick individuals (27). In a study by Reuben et al. on the attitudes of the medical students towards the elderly, they found that students had a negative attitude towards the elderly ("The elderly cannot adapt to changes", " the elderly constantly get sick", "the elderly are angry" (28). In some studies, quite the opposite results to these studies were obtained. For instance, in a study by McConatha et al. on the attitudes of the students at Turkish and USA universities towards getting old, it was found that the students had positive attitudes towards getting old. Moreover, in the same study, it was found that the Turkish students stated that they enjoy spending time with older people, visiting elderly relatives and helping old people more than students in the USA (29). In a study by McKinlay and Cowan on the attitudes of nursing students towards older patients/persons, it was found that students had positive attitudes towards older patients/persons (30).

In our study, the positive ageism and total AAS scores were found to be significantly higher in men than in women. In the study by McConatta et al. on students at Turkish and US universities, it was found that girls had more negative attitudes than boys towards older people (29). This finding is similar to our findings in this study. It is supposed that this finding obtained from the study results from various factors such as urbanisation, an increase in the numbers of nuclear families, the increased participation of women in the labour market or the fact that women care more about physical appearance than men do. The development of such negative attitudes by women may be related to the fact that the women spend more time with older people and they have to look after them more than men.

Although there was statistically no significant difference between the age groups and the mean AAS sub-dimension and total scores, the mean AAS sub-dimension and total scores of the respondents aged 41-48 were higher than those of the respondents aged 18-24 years old. In the study by Thomas and Hallebone on the attitudes of 11 and 15 year old students towards older persons, it was found that 15 year old students had more negative attitudes towards older people than 11 year old students (25).

In a study on the differences of opinions of men and women on ageism, both young and older people were included in the sample. In the study, male and female university students aged over 25 and men and women over 70 years of age were asked about their opinions about old age. It was concluded that old and young men scored young age as a more positive periods while they scored old age as a more negative period. However, there was a dramatic difference in the opinions of young and old women. Young women scored the period of old age as negative while old women scored it as positive (39).

When people are in their late adulthood, the next period in the course of their life is old age. Therefore, it may be expected that these people will be more sympathetic towards the elderly and perceive old age differently as they themselves will soon reach it. On the other hand, it may be more difficult for the adolescents and young adults to understand the elderly and to show sympathy towards them.

In our study, the limitations on the life of the elderly scores were found to be significantly higher in respondents with primary and high school education. Although it is expected that education will have a positive effect on developing a positive attitude towards the elderly, we were surprised to find that the scores of the respondents with primary and high school education were even higher than those of university graduates, whereas it is understandable for them to get higher scores than those without any education. It can be said that factors other than educational level may be determinant in developing such attitudes and, in particular, cultural tendencies are important.

In our study the total AAS mean scores of the respondents living in an extended family were found to be significantly higher than those of respondents who lived in a nuclear family. This

finding differs from the findings of Yilmaz et al. (13). This may be explained by the fact that an old person should be respected and cared about in an extended family and the process one can learn and acquire knowledge from the experiences of old people. Besides this leads us to believe that despite the rapid transformations in not only industrialisation and urbanisation, but also in economic and social structures, old people still maintain their importance in the family.

The total AAS scores of the respondents who were first degree relatives of the elderly family member were found to be significantly higher than those of respondents who were not. This is the result of a sense of duty or loyalty and a sense of responsibility required by their kinship.

There was no significant difference between the income levels and the AAS total mean scores of the respondents. This finding is similar to that of Yilmaz et al. (13). We can say that these results from the fact that Turkish culture includes such behaviours and attitudes as protecting the elderly, undertaking their care and the belief that an elderly person is not considered as an economic burden by other family members. In other words, the approach to an old person is largely related to emotional and behavioural dimensions.

The attitudes of the respondents with children towards ageism were more positive than those without. However, there was no significant difference between the groups. It may be possible for the respondents with a children that being a parent is accompanied by many characteristics such as compassion, mercy, responsibility, sharing etc. and they may also behave in such a way thinking that his/her attitude to the elderly today may in the future be adopted by their own children towards him/her.

Although the AAS scores according to the time spent with old person/persons in the same house were positive, there was no significant difference between the time spent together and the total AAS mean scores. In a study by Scot et al. on secondary and high school students, they found that students who met with their grandparents at least once a week had more positive attitudes towards the ageing process (35). This result in our study makes us think that the relatives who share the same house with old persons are in positive contact with the elderly and they benefit from their knowledge and experience. Nevertheless, always being together with an older person may lead to tedium especially in cases which require a lot of care; this in turn leads to the development of a negative attitude. In fact, the children of an older person may share the duty of taking care of their parents for certain periods.

There was no significant difference between the groups concerning ageist attitudes towards the elderly in the work force. In fact, it had been expected that the attitude towards elderly people in the work force would be positive, for in this case the old person is considered as a productive and consuming member just like the other members of the family.

Although the score medians of the respondents who lived with an elderly person in bad health were higher than those of respondents who lived with a healthy old person, there was no significant difference between the groups. This result obtained from the study is thought to stem from the fact that the family members want to support elderly relatives to make them feel healthy and happy in spite of their having experienced some physiological, biological and psycho-social losses in the natural course of their lives. On the other hand, taking care of a sick person may lead to negative attitudes over time (especially in cases of chronic diseases and those requiring a lot of care such as Alzheimer) as it will cause tedium.

Consequently, it was found that the relatives of elderly people had positive attitudes towards ageing and ageism. Healthy ageing is a concept that includes physical, psychological and social well being. Therefore, the attitude and support of the family and relatives of an elderly person are highly important for an older person in order to survive this course healthy. To determine the attitudes towards ageism of an older person's family members, who are an important social and emotional support for the elderly, is important in that their awareness can be increased in the subjects they are responsible for such as the support of their parents as they get older, and meeting their needs and requirements and they can it also have the change their incorrect or inappropriate attitudes towards ageism.

References

1. *Bilir N. Demographical Characteristics of the Elderly in Turkey and World (Editor Ariogul S,). Geriatrics and Gerontology. 1st Ed.. Ankara: MN Medikal & Nobel; 2006. p. 3-11.*

2. *Erdemir F. The importance of life quality in healthy old persons and ageing, Ist National Geriatrics Congress Congress Book. Turkish Geriatrics Foundation 30 October- 3 November 2002; 112-116.*

3. *Unalan T. The status of old age population in Turkey. The Turkish Journal Of Population Studies, Hacettepe University Press, Ankara. 2002; 22: 3-22.*

4. *Hacettepe University Population Studies Institute, Turkey Population and Health Survey Ankara: Hacettepe University Population Studies Institute 2008.*

5. *Republic of Turkey State Statistical Institute of Prime Ministry, The Population and Development Indicators,Population and Demography. http://tuikapp.tuik.gov.tr/nufusmenuapp/menu.zul Access date: 6th June2012).*

6. *Akgun S, Bakar C, Budakoglu. Tendencies of the old aged population in Turkey and World, Problems, Suggestions Journal of Turkish Geriatrics 2004; 7(2): 105-110*

7. *Adak, N. Informal Caregivers of the Old Persons: The Women Family and Society. Journal of Education, Culture and Research; 2003; 2(7): 81-89.*

8. *Gokce-Kutsal Y. Aging People of the Ageing World . Geriatrics, Ankara: Publishing of the Turkish Union of Doctors, 2007; 13-19*

9. *Civi S, Tanrikulu MZ. An Epidemiological Study on the Prevalence of Chronic Diseases and the Level of Physcal Incompetence and Dependency in the Elderly, Journal of Turkish Geriatrics 2004; 3(3): 85-90*

10. *Esen A. Health Problems in Old Age. Journal of Ege University Nursing Vocational High School, 1992; 8(3): 97-105.*

11. *Akdemir N, Cinar Fİ, Gorgulu U. The Perception of Old Age and Ageism. Turkish Journal of Geriatrics 2007; 10(4): 215-222.*

12. *Arasil T, Gokce-Kutsal Y. New Horizons in the Physical Medicine and Rehabilitation, Health of the Elderly, Gunes Medical Bookstore, 2010. p:1*

13. *Yilmaz E, Ozkan S. Attitudes of Nursing Students towards Ageism. Journal of Maltepe University Nursing Science and Art, 2010; 3(2): 35-53.*

14. *Cilingiroglu N, Demirel S. Old age and ageism. Journal of Turkish Geriatrics 2004; 7(4): 225-230.*

15. *Robinson B. Ageism. 1994 htpp://socrates.berkeley.edu/~aging/ ModuleAgeism.html (Erişim:12 Kasim2011).*

16. *Vefikuluçay D. Ageist attitudes of the University Students, Doctorate Thesis, Hacettepe University; Ankara 2008.*

17. *Cheung C, Chan C, Lee J. Beliefs about elderly people among social works and the general public in Hong Kong. Journal of Cross-Cultural Gerontology, 1999; 14(2): 131-152.*

18. *Minichiello V, Browne J, Kendig H. Perceptions and consequences of ageism: views of older people. Aging and Society; 2000; 20: 253- 278.*

19. *Polizzi KG, Millikin J. Attitudes toward the elderly: Identifying problematic usage of ageist and overextended terminology in research instructions. Educational Gerontology, 2002; 28: 367-377.*

20. *Taylor P, Walker A. Employers and older workers: attitudes and employment practices. Ageing and Society, 1998; 18: 641-658.*

21. *Walker H, Grant D, Meadows M. Cook, I. Women's experiences and perceptions of age discrimination employment: implications for research and policy. Social Policy & Society, 2007; 6(1): 37-48.*

22. *Ozdemir O. Ageist Attitudes of the University Students who Study. Master's Thesis. Ankara: Gazi University; 2009.*

23. *Soyuer F, Unalan D, Guleser N, Elmali F. Ageist Attitudes of the students at the Health Vocational School and the relation of these attitudes to some demographical variables. Journal of Mersin University Health Sciences, 2010; (2) 3: 20-25.*

24. *Oz F. The last stage of life: Elderly the review of psychosocial dimension. Journal of Kriz 2002; 10(2): 17-28.*

25. *Thomas T, Hallebone E. Adolescents' attitudes towards older people and their relationships with grandparents. Lincoln Papers in Gerontology No.30. Melbourne: Australian Research Council, Key Centre in Gerontology, La Trobe University: 1996.*

26. *Kotzabassaki S, Vardaki Z, Andrea S, Parissopoulos S. Student nurses' attitudes towards the Care of Elderly Persons. A Pilot Study. ICUS NURS WEB J 2002; 12: 1-9.*

27. *Moyle W. Nursing students perceptions of older people continuing society's myths. Australian Journal of Advanced Nursing 2003; 20(4): 15-21.*

28. *Reuben DB, Fullerton JT, Tschann JM. Attitudes of beginning medical students toward older persons: A five-campus study. The University of California Academic Geriatric Resource Program Student Survey Research Group. Journal of the American Geriatrics Society, 1995; 43(2): 1430–1436.*

29. *McConatha JT, Hayta V, Rieser-Danner L, McConatha D, Polat TS. Turkish and U.S. attitudes toward aging. Educational Gerontology, 2004; 30(3): 169-183.*

30. *McKinlay A, Cowan S. Student nurses' attitudes towards working with older patients. Journal of Advanced Nursing, 2003; 43(3): 298-309.*

31. *Palmore EB. Research note: Ageism in Canada and the United States. Journal of Cross-Cultural Gerontology, 2004; 19(1): 41-46.*

32. *Pampel CF. Aging, Social Inequality and Public: 1998.*

33. *Haken JDT, Woolliscroft JO, Smith JB. A longitudinal investigation of changes in medical students' attitudes toward the elderly. Teaching and Learning in Medicine, 1995; 7: 18-22.*

34. *Kite ME, Stockdale GD, Whitley BE, Johnson BT. Attitudes toward younger and older adults: an updated meta-analytic review. Journal of Social Issues, 2005; 61(2): 241–266.*

35. *Scott T, Minichiella V, Browning C. Secondary school students' knowledge of and attitudes towards older people: does an education intervention programme make a difference? Ageing and Society, 1998; 18: 167-183.*

36. *Slevin OD'A. Ageist attitudes among young adults: implications for a caring profession. Journal of Advanced Nursing, 1991; 16(10): 1197-1205.*

37. *Mosher-Asley PM, Ball P. Attitudes of college students toward elderly persons and their perceptions of themselves at age 75. Educational Gerontology, 1999; 25(1): 89-102.*

38. *Loretto W. Duncan C, White JP. Ageism and employment: controversies, ambiguities and younger, people's perceptions. Ageing and Society 2000; 20: 279-302.*

39. *http://www.webster.edu/~woolflm/ageism.html. erişim: 21.05.2012*

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Traditional Turkish culture applications at baby - child care

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Abstract

The aim of this study is to bring out the traditional beliefs about the baby-child caring and contribute to the literature on that issue.

Metods: The study was done descriptively. Research phase consists of mothers living in İstanbul and sample consists of 460 mothers having at least one child and accepting to join. As for the gathering of data, an 11 item questionarre was used by the researchers. The items question the applications for the well being of baby, belly caring, rash caring, colic caring, toilet education, sleep patterns and the applications for the seperation of the baby from the nozzle.

Finding: At this study, traditional applications base on beliefs such as not going out for 40 days or not letting people kiss the baby are among the most frequently expressed applications. 21.7%. Apart from these; existence of giving alms, amulet, praying are found out.

Conclusion: We are glad to notice that traditional harmful applications are very few in our study. It is one of the ethical principles of nursing to respect individual's or family's cultural or religious beliefs. It is also necessary for a nurse's education-counselling role to know her societies' harmful or unharmful traditional applications.

Introduction

Cultur, beliefs and traditions are parts of life of a society. The place of traditional culture on the issues like health, illness and child care is always exist as well as many other reasons. That belief and traditional applications might be sometimes logical and unharmful and sometimes illogical but harmful.

People don't experience the traditional applications just for the sake of they are belief or tradition. They make these applications live as far as they make sense for them. These applications often make an individual feel himself better and safe. It is a fact that altough traditional applications exist all around the World, they are applied more frequently in undeveloped and developing countries (1).

Turkish society with its 2200 yeared inheritance made up a rich folklore by blending the culture of the region they settled with the migratory culture of their own. For that reason, in different parts of Anatolia, as well as different kinds of applications, similar and totally the same applications are come across (2).

Even though rapidly increasing and changing innovations at medicine, some ineradicable beliefs stil exist in the life of societies (3). Soieties' cultural beliefs and applications should be known by the nurses whose target groups are the individual and his family. Actually, the whole culture, beliefs and applications which effect the child's health are important since they determine the quality of nursing care. On the other hand, approving the applicability of unharmful beliefs is important for the principle of respect. People need to be quit the applications which can be harmful for both the child and the society.

The aim of this study is to bring out the traditional beliefs about the baby-child caring and contribute to the literature on that issue.

Materials and Method

The study was done descriptively and data were gathered between December 2009 and February 2010. Research phase consists of mothers living in İstanbul and sample consists of 460 mothers having at least one child and accepting to join.

As for the gathering of data, an 11 item questionarre was used by the researchers. The items question the applications for the well being of baby, belly caring, rash caring, colic caring, toilet edu-

cation, sleep patterns and the applications for the seperation of the baby from the nozzle.

The size of the sample, when the woman population in Turkey is taken into consideration, is found out as 384 with 5% tolerance and 95% confidence interval and 460 mothers participated into the study. The answers were categorized and frequency distribution was made.

Result and Discussion

Postpartum period is crucial for both the mother and the baby. It requires an accordance for all family members to new situation physchlogicaly, sociologically, biologically. It is so important to support the mother and baby at this hard period. Not having enough education, not reaching health services, or giving birth accoring to beliefs direct people to traditional beliefs.

At this study, traditional applications base on beliefs such as not going out for 40 days or not letting people kiss the baby are among the most frequently expressed applications. 21.7%. Apart from these; existence of giving alms, amulet, praying are found out (Table 1).

Evi leye can be defined as bade ye from a stranger and it is very common among cultures to protect baby and mother from the evil eye. Wearing blue beads, praying, staying away from strangers are among the widely known applications (1).

Similar to our study, Beşer et al.(4) state that wearing amulets, praying are the most common applications to protect mother and baby from evil eye. (54.3%). According to Gölbaşi and Eğri (5) the most frequently used traditional application at the postpartum period is not letting the baby and the mother step out for 40 days. (91.5%).

Most of the mothers behave similarly on traditional applications in the literature. For instance; not stepping out for 40 days, praying, using yellow cloth to protect from hepatite, applying honey and salt to his skin, applying cord blood to the face of baby) (1,4,5,6,7,8,9)

It is known that in India and China and Iran, mother and the baby are not allowed to step out for 40 days. After 40 days a special ceremony is held for them (1). In India the local "duis" are responsible for the caring of baby. In order to protect the baby from the evi leye Indian women make a black point under his bed; the baby sleeps with his mother for 40 days and some metal materials are placed under his bed to protect him from evil eyes (10).

In our study, traditional applications are similar to other studies. It is good not having applications risky for the baby's health but applying salt to his skin. Moreover; accepting breastmilk as the primary factor which protect the baby's health can be construed as another positive result.

At table 2 using traditionally applied material for belly caring has a rate of 11%. The rate of powders (coffee, ash, powder, salt) used for belly caring is 4.1% and liquids (salty water, olive oil, grape juice) has the rate of 6.9%.

Dinç (7) states in his study, which is similar to ours, that the rate of using coffee,salt,olive oil is 21.5%, powder 36%, the rate of those mother preffering dry caring is 31%. Bölükbaş et all. (6) points out that 19.7% of mothers use batikon, 6.4% of them keeps it dry and clean, and 2% of them uses breast milk.

In other similar studies it is stated that mothers apply olive oil, powder, coffee, clay, ash, milk to their babies' belly (8,9,11,12). It is also reported that in Nepal and Bangladesh mothers use mastard

Table 1. The applications for the well being of the baby

Tihngs which are done for the baby's being healthy and for keeping him away from diseases	N*	%
Feeding with breast milk	224	48.7
I have his vaccine in time	109	23.7
I protect him from cold and keep him warm	126	27.4
I dont go outside for 40 days I let noone touch or kiss him	100	21.7
I cover him with a yellow cloth and apply olive oil to his body (to protect him from hepatite)	20	4.3
I apply honey to his body (for a good skin) and wash him with salt or salty water. (To avoid smell of sweat)	57	12.4
I Apply some holly ventures to protect from badness. (drinking holly water, praying,)	13	2.8

**N is replicated*

oil for belly caring (13,14). In Etihopa 48.7% of mothers use butter for belly caring (15).

Since the umbilical is accepted as a part of baby in Turkish Culture, it is related with the future of the baby. Keeping or burying the falling umbilical is a common tradition. The falling umbilical can be kept inside the home for the child's being loyal to his family or it can be buried to the garden of a school, university, hospital for child's having a good job.

In our study, the rate of keeping the falling umbilical is found out as 28.9% and its burying 55% (Table 2). Also, a lot of other studies include such beliefs as keeping or burying the falling umblical (1,6,9).

Skin problems are often experienced for newborns. It is recommended that in order to prevent bez dermatitin, that area should be cleaned up with water and cotton, ventilated, and zinc oxide should be used. Using powder, creams including antibiotics, creams including vitamine A are not recommended since they are proof based applications (16).

Olive oil and many other vegetable tallows are used for baby's skin care in different cultures. So that; Association of Women's Health Obstetric and Neonatal Nurses (AWHOWN) recommends that the type these tallows should be assessed. It is shown in Dharmstadt and Saha's (17) study that mastard and olive oil reduce skin barrier functions and increase transdermal fluid loss. In addition; it is shown in the same study that sunflower oil and saffron oil reduce the transdermal fluid loss and increase the skin barrier functions.

In our study the rate of using another method except from prof based ones is determined as 33% powder, 10% olive oil and 1.5% others (cologne, soil, salty water) (Table 2). Similarly, in many other

Table 2. Applictions for falling belly to cure the rush and colics

The applications which are done for the falling of belly	N*	%
I do nothing and waiting for its falling	106	23
I just keep it clean and dry	91	19.8
I use the recommended solution (betandin, alcohol)	160	34.8
I shed some kind of powders (coffee, salt,powder)	18	4.1
I apply some liquids (olive oil, ice, breast milk, cream, salty water)	32	6.9
No answer	53	11.4
Total	**460**	**100**
The applications which are done for the falling part of the belly		
I keep it	133	28.9
İ throw it away	51	11.1
I bury it (into school garden,grave yard, a mosque)	253	55
No answer	23	5
Total	**460**	**100**
The applications which are done when the baby has rash		
I used powder	152	33
I used olive oil	48	10.4
I used rash cream	236	51.3
I used moustirizing	75	16.3
I washed and ventilated	167	36.3
Others (cologne, salty water, soil,egg)	7	1.5
***N is replicated.**		
The applications which are done when the baby experience throes of fart		
I applied massage to his belly	85	18.5
I kept his belly and feet warm	18	3.9
I used herbal tea and powders (daisy,cumin,mint,lemon.olive oil, coconut)	63	13.7
I stroke his back and massaged	329	71.5
Others (shower, leg exercises, applying soup)	43	9.3
***N is replicated.**		

studies most frequently used traditional methods are expressed as powder, olive oil, butter and soil with the rates ranging 12-56%. (6,7,8,9,11,12,18). For that reason it can be recommended that mothers should be informed on the using of powder, olive oil, and soil.

Colic is a common state occurs after 3 months from the birth with excessive crying. It is a hard and tiring process for both mother and the baby (19,20).

In our study, 71.5% n= 329 of mothers answered the question "what did you do when your baby experienced colic?" as "massaged on his back" and 18.5% n= 85 of them as massaged on his belly and 13.7% of them as used herbel tea. Çalişkan and Bayat (11) point out in their studies that 84.7% mothers used metsil, 13.8% of them used herbal tea and 1.9 % of them drink the baby olive oil. The rates of using herbal tea are so similar to our study.

Even though there is not a certain treatment for Infant Colic, there are some recommendations to relieve the baby. For example; ritmic exercises, keeping him warm, decreasing of stimulants, using teat, listening to music, massage to back, herbal tea etc can relieve the baby (19,20). In the study no harmful application for baby's health are come across.

Toilet education is very important for the child who start to step. The phsyological control skill of Anal and uretral sifinkters are acquired after the child starts stepping. Furthermore, physco-phisyological readiness doesnt complete before 18-24 months (21).

During the toilet education parents should be in accordance with the child and they should be aware of child's readiness symptoms. There are some cultural differences on the starting of the education. Altough the use of potty and toilet changes from person to person it is know that child feel himself safe when he use a potty. Punisment should be avioded in toilet education. Child should be encouraged. It is recommended to explain the use of potty with the help of audio-visual materials (21).

Table 3. Applications on Toilet education

Starting the toilet education	**N**	**%**
Before 1 year	84	18.3
Between 1.5-3 years	306	66.5
After 4 years	7	1.5
When he is ready	34	7.4
As soon as he was born	1	0.2
No answer	4	0.9
The method used for toilet education		
I have no special application	20	4.3
I explain it	67	14.6
I often take him to toilet	27	5.9
I use a potty.	69	15
I take him to toilet at particular times	114	24.8
I use games	39	8.5
I use reward technique	59	12.8
I go to toilet together with him	50	10.9
I do not fasten his diaper	27	5.9
***N is replicated.**		
The applications when he is pissed off during the process of toilet education		
I clean it up without being angry	229	49.8
I make him embrasse,and leave it dirty	46	10
I warn and frigten him	124	27
I wait patiently	25	5.4
I punish him	28	6.1
No answer	8	1.7
Total	**460**	**100.0**

It can be accepted that more than half of the mothers participating in our study have adequate knowledge on toilet education since they approve starting of education between 1.5 – 3 years 66.3%. However, 43% of mothers use the way of embrassing or frightening the child when he pissed off. (Table 3). So that it can be concluded that mothers need more information on that issue. In addition; mothers' expressing frightening, being angry, punishing seperately can be seen as they do not think all of them as punishment.

In cultural applications timing and method are the crucial parts of toilet education. For example; a Chinese family is so free about timing, method is special to child. Child wears diaper during his chilhhod. When he starts stepping , his parents opens a space between his trousers leg. This can last till the child is 5 years old (21).

Most of growth hormones are ecreted during sleep. For that reason a qualified sleep accelerates the brain development and makes the baby grow healthly. But the very first years of life are the most problematic years for a good sleep.

In our study, 50.2%of mothers answered the question of "what did you do to put the baby asleep?" answered as "I sang a lullaby" , 42.6% n=196 of them as "I put him on my lap" and 14.1% n= 65 as told him fairy tale and 11.3% n= 52 of them as" I used teat." In their study Bölükbaş et all. (2009) found out that 33% of mothers put their babies on their feet , 15.8% of them sing a lullaby and 5.4% of them give teat with honey to their babies. As it can be seen in our study, it is a very common application to sing a lullaby or put him on the lap in Turkish Culture.

A lullaby is a kind of poem singing in a rhytmic way. The mother shows her love, compassion to her baby with lullabies. Lullabies are also important for child's language,cognitive,emotional development. It is known that lullabies improve the child's speaking skills (22).

American Pediatri Academy (23) and WHO recommend that babies should be fed only by breast milk first 4-6 months and the breast milk should be given to the child together with supplementary food till the age of 1. In our study , 48.7% of mothers state that they breastfeed their child till the age of 1 and 21.3% of them till the age of 2. (Table 4). According to TNSA report in 2008, 56.6% of Turkish mothers breastfeed their baby till 1 year old and 25.3% of them till 2 years old. Data in our study are similar to TNSA report.

In our study, a traditional application that is; breastfeeding the baby until a new pregnancy, is found out as 0.7% n=3. (Table 4). According to beliefs of Muslim society, it is forbidden by religion to breastfeed the baby if the mother knows she is pregnant. Because the milk belongs to new

Table 4. Applications for breast milk and seperation from the nozzle

The time of seperating the baby from the breast milk	**N***	**%**
He left when the milk finished	63	13.7
After six months	50	10.9
Between 1-2 years	224	48.7
2 years and afterwards	101	21.9
Until a new pregnancy	3	0.7
Until he left it himself	19	4.1
***N is replicated.**		
The applications for seperating the baby from the nozzle		
I do nothing.	68	14.8
I start formulated milk and increase the supplementary food.	83	18
I use teat+candy	4	0.9
I use teat+baby bottle	66	14.3
I apply frigthening and disgusting things to my nipple. (hot pepper, garlic, vinegar, ash)	140	30.4
I decrease the breastfeeding and make him forget	71	15.4
I send him away somewhere.(to grandparents)	16	3.5
No answer	12	2.7
Total	**460**	**100**

born. (Elmaci and Özelçi 2007-www.antropology.net.) But the rate of that harmful application in our study is too low.

Seperation of the baby from the nozzle can change from baby to baby. If the mother decide that it can harder. The recommended method is to seperate the baby from the nozzle gradually. So that both mother and the baby gain time in that process. In our study when the mothers are asked what the did to seperate the baby from the nozzle, 30.4% n=140 of them expressed that they applied some materials to their nipples, and 3.5% of them expressed that they sent the baby to grandparents (Table 4).

In Çalişkan and Bayat's (11) study, applying some materials to nipple is found out as 6.7%. in other studies about traditional applications, some data about first breast feedin or first food take place. The most frequently used applications are feding the baby with sugary water, throwing first milk (7,8,11,12,24).

Conclusion and Recommendations

WE are glad to notice that traditional harmful applications are very few in our study. It is one of the ethical principles of nursing to respect individual's or family's cultural or religious beliefs. It is also necessary for a nurse's education-counselling role to know her societies' harmful or unharmful traditional applications. In educational or intern programmes on health sciences it can be recommended that emphasis should be on the respect of individual's cultural,religious beliefs which are not harmful for individual's or societies' health.

References

1. *Eğri Bozkuş G, Konak A. Traditional belief related to postpartum period and samples for practices from Turkey and the World. Journal of Word of Turks, 2011; 3(1): 143-155.*
2. *Özyazicioğlu N, Öncel S. Traditional approach in child care, In: Intercultural Nursing, edited by Tanriverdi G, Seviğ Ü, (Istanbul Tip Publication, Istanbul,Turkey), 2011: 203-238.*
3. *Karabudak SS, Yavuz B, Yilmaz BH, Başbakkal Z. Traditional practices of mothers' abouth child health. Journal of Istanbul University Florance Nigtingale School of Nursing, 2009; 17(3): 190-197*
4. *Beser A, Topcu S, Coşkun A, Erdem N, Gelişken R, et al. Traditional child care practices amoung mothers with infants less than 1 year old. Dokuz Eylül University School of Nursing Electronic Journal, 2010; 3(3): 137-145*
5. *Gölbaşi Z, Eğri G. Traditional practice to mother care in postpartum period. Cumhuriyet Medical Journal, 2010; 32: 270-282*
6. *Bölükbaş N, Erbil N, Altunbaş H, Aslan Z. Traditional practice about child care of mothers who owner 0-12 mounth baby. International Journal of Human Sciences, 2009; 6(1): 164-176*
7. *Dinç S. To determine the traditional practice which mothers, having 0-1 years old children, registered to health center no:4 in ŞanliUrfa Center. Turkish Journal of Research and Development in Nursing, 2005; 1(2): 53-63*
8. *Biltekin Ö, Baran OD, Denkli MD, Yalçinkaya S. Prenatal period and infant traditional practice that mothers having 0-1 years old child in Naldöken Statement. Sted, 2004; 3(5): 166-168.*
9. *Yalçin H. The traditional practice related pregnancy, childbirth, puerperality, and child care among women in Karaman, Turkish Pediatric Journal, 2012; 55: 19-31.*
10. *Chouldbry UK. Traditional practice of women from India: pregnancy, Childbirth and newborn care. JOGNN, 1997; 26(5): 533-539*
11. *Çalişkan Z, Bayat M. Baby care application of mother and acting faktors of these applications: a sample on Kapadokya, Journal of Anatolia Nursing and Health Sciences, 2011; 14(2): 23-30*
12. *Eğri G, Gölbaşi Z. Traditional postnatal infant-care practices of 15-49 years old married women. TAF Preventive Medicine Bulletin, 2007; 6(5): 313-320*

13. *Thatte N, Mullany LC, Khatry SK, Katz J, Tielsch JM, et al. Traditional birth attendants in rural Napal, knowledge,attitudes and practices abouth maternal and newborn health. Glob Public Health, 2009; 4(6): 600-617.*

14. *Alam A, Asraf Ali N, Sultana N, Mullany LC, Teela C, et al. Newborn umblical cord and skin care in sylhet distric, Bangladesh: implication for promotion of umblical cord cleansing with topical chlorhexidine, J. Perinatol, 2008; 28(2): 164*

15. *Girma T, Nida H. Traditional newborn care in Jimma town, Southwest Ethiopia. Ethiop J. Health Scences, 2008; 18(3): 79-86.*

16. *Association of Women's Health Obstetric and Neonatal Nurses (AWHONN). Neoanatal Scincare Second Edition. Evidence Based Clinical Practice Guidelin. 2007.*

17. *Dharmstadt G, Saha Samir K. Traditional practice oil massage of neonates in bangladesh. Journal of Health population and Nutrition, 2002; 20(2): 184-188.*

18. *Özen M, Özgör B. Höllük:an Anatolian reality. Inonü University Medicine Faculty Journal, 2006; 13(2): 131-133*

19. *Çetinkaya B, Başbakkal Z. The effectiveness of aromatherapy massage using lavender oil as a tratment for infantile colic. International Journal of Nursing Practice, 2012; 18: 164-169*

20. *Hall B, Chesters J, Robinson A. Infantile colic: a sistematic rewiew of medical and conventional therapies, Journal of Pediatrics and Child Health, 2012; 48: 128-137*

21. *Wong DL, Hockenberry MJ. Health promotion of the toddler and family, In:Wong's Nursing Care of Infants and Children, edited by Wilson D, Wilkelstein M, Kline NÇ, (Mosby, St Louis), 2003; 603-608.*

22. *Güneş F. Effects of lullabies on linguistic and mental development of children. Journal of Word of Turks, 2010; 2(3): 27-38.*

23. *American Academy of Pediatrics (APA). Pediatric Clinical Practice Guideliness and Policies. 4th ed. 2004.*

24. *Eker A, Yurdakul M. The knowledge and practices mothers to related of baby feding and breastfeeding. Sted, 2006; 15(9): 158-163.*

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Influence of surgery and orthoptic pleoptic therapy on functionality of binocular eyesight

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Abstract

The purpose of this research is to examine at what age a surgery and orthoptic pleoptic exercises create better conditions for binocular eyesight improvement.

The research covers 58 examinees with esotropia who underwent a surgery. Data used herein were collected on the basis of history data of examinees treated at Department for orthoptic and pleoptic of the Clinic for eye diseases, University Clinical Center Tuzla. Their age is from 3 to 14. The research includes three period of observation: first – initial examination, a period after surgery and a year after surgery. The examinees were divided into two age groups: 3-6 and 7-14. SPSS 17.0 for Windows program package was used for data processing applying analysis of a variant (ANOVA), i.e. p-value, hi-square test (dependence test) and regression analysis.

Upon completion of data processing, taking into consideration results obtained, it may be concluded that better results were reached at younger age examinees, aged 3-6, expressed in decrease of strabismus angle size and higher percentage presence of binocular eyesight a year after surgery, in simultaneous perception and fusion, in comparison to 7-14 age examinees.

None examinee of both age groups had stereovision. All these factors represent favorable conditions for further orthoptic pleoptic therapy of children.

Key words: esotropia, age, visual acuity, strabismus angle size amblyopia, binocular eyesight, orthoptic pleoptic therapy.

Introduction

Eyesight is very important for life and work of a human being. Thus, eyesight deterioration takes special place (1). Sensory and perceptive capacities refer to capacities for reception and interpretation of information from environment or from proper body (2). Knowledge of real eyesight acuity is very important at children in development and early childhood, but what is more interesting and practical for an ophthalmologist is comparison of eyesight acuity among the two eyes at that age (3). Eyesight acuity is capacity of distinguishing details in space, i.e. clarity or acuity of eyesight (4). Binocular eyesight is not innate but gradually developed from birth till the age of 5-6. If any deterioration appears prior to this age, it will be modified by incomplete development process. This is why adaptation to creation of new mechanisms on anomalous motoric conditions appears (5). Binocular vision deteriorations are often connected to deterioration of operation of visual centers in cortex due to specific flow of information, which is related to difficulties in maturation of eyesight path (6). Early and permanent diagnostics of different visual functions is necessary for the purpose of prompt therapy and prevention of serious pathological changes on visual device and problems in vision operation in general. "Squint as far as esthetics concerns may be improved at both 5 or 75, but decrease of eyesight on squint eye can be and must be stopped at early childhood. Starting school may be late" (7).

Convergent strabismus is the most frequent type of squint eyes, especially at children. The noted incidences vary from 20% through 57%. Analyzing scientific researches, a significant presence of esotropia may be noted which varies from 90% through 96%, while egsotropia varies from 0-8% cases (8). The most frequent is V esotropia, then follows A estoropia, V egsotropia and A egsotropia. The V type is present in about 60% cases, while A form of strabismus in 36% of cases (9). Patients with A and V syndromes complain about asthenopia

disturbances and double images, especially when working nearsight and have a forced head position. It is important to make a precise clinical examination in order to reach a decision regarding proper treatment (10). Characteristics of strabismus at child age are motor anomaly or non parallelism of vision axes (11). Amblyopia is frequent, too, which may affect 5% of total population. The most frequent amblyopia characteristics are refraction anomaly, strabismus, nystagmus and ARK. Early detection of amblyopia shortens duration of defectologic-medical treatment (12). In the UK, there are screening programs for identification of children (possibly) suffering from amblyopia (13). Treatment of amblyopia at early age depends on early detection of strabismus and amblyopia, i.e. on prompt and good prevention thereof (5). If parallelism of vision axes may be reached and maintained without surgery, like in case of accommodation esotropia, then it is not necessary to perform a surgery. Surgery on bulbomotors improves esthetic appearance and position of pupil (14). After surgery, new motor and anatomic conditions are established which replace previously established sensor capacities. Thus, in post surgical period it is necessary to ascertain desired sensor qualities applying orthopleoptic methods and not permit creation of new anomalies, including amblyopia and double images (15).

Aim of research

To examine at what age a surgery and orthoptic pleoptic therapies give best results for functional improvement of binocular eyesight.

Method of operation

Sample of examinees

The research includes 58 examinees, 30 females and 28 males. They are 3 to 14 years old.

In order to determine at what age a surgery and orthoptic pleoptic exercises provide best conditions for reaching binocular eyesight, examinees were divided into two age groups: 3-6 year olds and 7-14 year olds.

Amblyopia (poor eyesight), as accompanying phenomenon, was present at 36 examinees, while 22 of them were without amblyopia.

Sample of variables

The research includes examination of multiple perception of characteristics. The examined independent variables are: age, eyesight acuity on the right and left eye, strabismus angle size, and amblyopia, while dependent variable is a binocular vision.

Mode of research conduction and instruments of measurement

Data used for this research were gathered on the basis of history of patients processed and treated at Department for orthoptic and pleoptic, Clinic for eye diseases, UCC Tuzla.

Criteria for data processing were examinees with esotropia (convergent strabismus), who were performed a surgery. The research was conducted in three periods of observation: the first (initial) examination, immediately after surgery and a year after surgery, including orthoptic pleoptic exercises. The following methods were used within the research: analysis of medical documentation, examination of eyesight acuity, refraction, examination of eye motility and occulomotor balance of eye muscle, cover-uncover test, measurement of objective deviation, i.e. of objective angle of strabismus (on synaptophore with obliged altering occlusion before measuring the angle), examination of binocular vision (on synaptophore).

Data processing methods

SS 17,0 for Windows program package SP was used for data processing.

For observed variables at each examination, for every group of examinees established according to their age, measures of central tendency, measures of dispersion and quartiles were calculated. To test presence difference of middle values among the groups established according to the age of patients (independent samples), and according to other criteria, analysis of variance (ANOVA), i.e. p-value, was applied. To see whether there is a statistically significant connection (interdependence) among some variables, a dependence test, i.e. Chi-square test was applied.

Results

According to results obtained and presented in table 1, the group aged 3-6, p-value was higher than 0,05 level of significance on which testing difference in proportions was performed, only in case of stereovision modality. Thus, there is no statistically significant difference in relative presence of that modality between the first – initial examination and examination after a year. In case of other two elements of binocular examination like simultaneous perception in connection with fusion and in case of fusion itself at the above mentioned age, p-value is higher than 0,05 level of significance on which testing difference in proportions was performed, only in case of stereovision element. Therefore, there is statistically significant difference in relative presence of that element of binocular vision between the first initial examination and examination after a year. This means that none of examinees had stereovision neither before nor after a surgery and post surgical recovery. In case of other two elements of binocular vision like simultaneous perception in connection with fusion and in case of fusion itself without presence of simultaneous perception at the above mentioned age, p-value is less than 0,05, and therefore there is a statistically significant difference in relative presence of elements of binocular vision between the first initial examination and examination after a year. So, these elements of binocular vision are more present in the period of a year after surgery, which means that examinees have reached simultaneous perception in connection with fusion a year after surgery. However, when it is about the group ages 7-14, the calculated p-value of higher than 0,05 level of significance on which testing difference in proportions was performed, stereovision and simultaneous perception with fusion modalities. Therefore, there is no statistically significant difference in relative presence of elements of those modalities between the first initial examination and examination after a year. When it is about fusion itself in the mentioned age, p-value is less than 0,05, and therefore there is a statistically significant difference in relative presence of that modality between the first initial examination and examination after a year. Thus, examinees have reached just fusion without simultaneous perception in the period of a year after surgery.

Discussion

The aim of surgery, i.e. of a surgical treatment of strabismus, is to put eyes in plane primary position and to enable binocular vision. Preoperative diagnostics of motor and sensor anomaly must be accurate. Small children with strabismus must be operated at early age in order to enable development of binocular vision. If strabismus at small children is present for a longer period of time, it is greater danger that sensor anomaly could persist through the whole life. A surgery is performed from the third to the sixth year of age, not later than starting school (5). The purpose of surgery in strabismus is to reach good function, i.e. binocular vision and esthetic result for psychological reasons (16). As above mentioned, surgical treatment is mostly indicated upon completed conservative therapy, i.e. orthoptic pleoptic therapy, where occlusion is first performed with appropriate correction (binoculars) and then treatment of amblyopia or improvement of vision on eye with poor sight. The purpose of surgery is to reach binocular vision and esthetic effect as a psychological problem. Upon surgical treatment which has helped gaining orthophoric position of eyes or almost orthophoric

Table 1. Binocular eyesight

Respondents by age groups	Number of respondents	Binocular eyesight	The first initial- review		Year after surgery		P
			f	%	f	%	
3-6 year	39	Simultaneous perception and fusion	0	0,00	5	12,82	0,017
		Fusion	0	0,00	11	28,21	0,000
		Stereovid	0	0,00	0	0,00	1,000
7-14 year	19	Simultaneous perception and fusion	0	0,00	2	10,53	0,135
		Fusion	0	0,00	4	21,05	0,024
		Stereovid	0	0,00	0	0,00	1,000

position, such condition may remain throughout the whole life, but may be changed, too.

Upon completed surgery, orthoptic pleoptic therapy is still performed at Department for orthoptic pleoptic, UCC Tuzla. Exercises for remaining amblyopia are performed, too, applying occlusion therapies, exercises for synaptophore, at what point fusion exercises begin as well as exercises of convergence for establishment of a stable simultaneous perception. Muscle and convergence exercises are continued just like stereogram exercises, all with the aim of establishing and strengthening binocular cooperation.

Conclusion

On the basis of obtained results of the research, the following conclusion may be reached:

Younger examinees, from 3 to 6 year olds, have reached better results in establishing simultaneous perception with fusion as first two elements of binocular vision in respect to the older age group of examinees, 7-14. This means that prompt preoperative preparation, surgery and orthoptic pleoptic therapy have had positive influence on gaining first two elements of binocular vision at younger examinees in comparison to the older ones, who were not included in treatment on time.

References

1. *Defterdarević-Serdarević T, Serdarević R. Dijete i razrookost, Sarajevo: Svjetlost, 1996.*
2. *Smiljanić N. Ispitivanje vidnih funkcija, Beograd: Zavod za udžbenike i nastavna sredstva, 2001.*
3. *Stankov B, Ljutica M. Monokularne i binokularne funkcije vida, Beograd: Istraživanja u defektologiji 7. CIDD, 2005; 179-190.*
4. *Ohlsson J. Ambliopia and Subnormal Visual Acuity-Studies of prevalence, assessment and outcome, Goteborg University Sweden: Departman of Ophthalmology Institute Of Clinical Neuroscience, 2003.*
5. *Čelić M, Dorn V. Strabizam i nistagmus, Zagreb: Medicinska naklada, 2004.*
6. *Golubović S i sur. Smetnje u razvoju kod dece mlađeg školskog uzrasta, Beograd: Defektološki fakultet, Univerziteta u Beogradu, 2005.*
7. *Parunović A. Upoznajte svoje oči, Beograd: Zavod za udžbenike i nastavna sredstva, 1997.*
8. *Kim J, Hwang JM, Kim H, Yu Y. Characteristic ocular finding in Asian children with Down syndrome, London: Eye Scientific Journal of the Royal College of Ophthalmologists, 2002; (16): 710-4.*
9. *Noorden GK, Campos E. Paralytic strabismus, IN: Noorden GK, Campos E, editors. Binocular vision and ocular motility. 6th ed. St. Louis: Mosby, 2002; 396-413.*
10. *Risović D, Vicić D. Alfabetski sindromi, Vojnosanitetski pregled, 2008; 65 (1): 57-60.*
11. *Lakoš-Krželj V. Strabizmi dječije dobi, Zagreb: Paediatr Croat, 2004; (48): 240-246.*
12. *Radžo Dž, Tulumović Š, Sarajlić Dž, Hundur M. Rani tretman ambliopije, Defektologija, 2008; 14 (1): 58-62.*
13. *Carlton J, Kaltenthaler E. Amblyopia and quality of life.Systematic review, Eye, London: Macmillan Publishers Limited UK, 2011; (25): 403-413.*
14. *Lakoš-Krželj V, Bojić L, Krstulović S, Karlica D, Kovačić Ž. Učikovitost jednostrane retropozicije kod ezotropije, Zagreb: Ophthalmol Croatica, 1997; (6): 51-9.*
15. *Helveston EM. The Origins of Congenital Esotropia, J Ophthalmol Strabismus, 1993; (30): 215-32.*
16. *Bradamante Ž. Oftalmologija, Zagreb: Nakladni zavod Globus, 1994.*

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Recurrent hemorrhage after Gamma Knife radiosurgery for periventricular arteriovenous malformations associated with an intraventricular aneurysm: a case report

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Abstract

We report a 58-year-old man who had gamma knife radiosurgery (GKS) treatment of periventricular arteriovenous malformations (AVM) associated with an intraventricular aneurysm. New hemorrhage occurred in the ventricle five days after GKS treatment. An emergency craniotomy was performed to clear the hematoma and resect the AVM with the aneurysm as well. The patient recovered well, just with a little hypomnesia nine months after the craniotomy.

Key words arteriovenous malformations, aneurysm, intraventricular hemorrhage, Gamma Knife radiosurgery

Introduction

The incidence of aneurysms coexisting with AVMs ranges between 2.7% and 16.7% [1-4], and the risk of hemorrhage is about 7% to 10% annually [3, 5]. It is more dangerous of hemorrhage in patients associate with AVMs and an aneurysm than just have AVM. The anatomical relationship between AVM and aneurysm is critical in deciding the best management. Stereotactic radiosurgery as a method of treating AVM has been widely accepted. Intranidal aneurysm or aneurysm located in the feeding artery proximal to the AVM had been cured successfully. We report a case of recurrent hemorrhage after GKS treatment of the intraventricular AVM and aneurysm.

Case Report

This 58-year-old right-handed man to our institution presented with a sudden onset of severe headache and vomiting. The patient was conscious without any abnormal cranial nerve functions. Computed tomography (CT) scan revealed ventricular system hemorrhage (Figure a). Seven years ago, the patient had been diagnosed with AVM after an intracranial hemorrhage by magnetic resonance angiography (MRA). Unfortunately they lost the MRA film. For some personal reasons, he had a conservative treatment instead of surgical or endovascular treatment. Cerebral DSA was taken to find the origin of the hemorrhage and it showed an AVM supplied by right anterior cerebral artery (ACA) with an aneurysm located at the branch of right ACA (Figure d). Enhanced magnetic resonance image (MRI) showing that the AVM bellowing and closing to the aneurysm (Figure b and Figure c). It was difficult to access and embolize these lesions by endovascular techniques and was believed to have a high risk of craniotomy for the location. After careful consideration the patient and his family decided to have a GKS treatment. After twelve days a GKS was performed, with a maximal dose of 36 Gy and a margin dose of 18 Gy to the AVM and aneurysm. Five days later, the patient presented sudden onset of headache, vomiting and fecal and urine incontinence followed by loss of consciousness soon. An emergency CT scan showed an intracranial hematoma in the same region (Figure e). An emergency craniotomy was performed to clear the hematoma and resect the AVM with the aneurysm as well. After craniotomy, the patient was sent to the intensive care unit for observation and follow-up treatment. The patient recovered well, just with a little hypomnesia nine months after the craniotomy (Figure f).

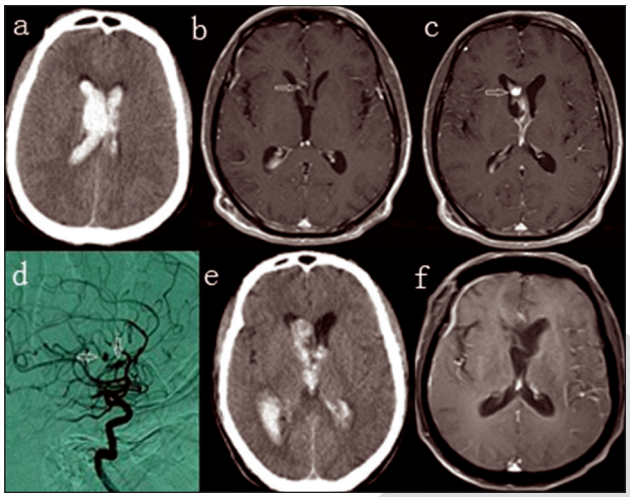

Figure 1 a. CT scan revealing bilateral lateral ventricle hemorrhage. b and c. Enhanced MRI showing that the AVM (b arrow) bellowing and closing to the aneurysm(c arrow). Both the AVM and aneurysm were locating at right lateral ventricle. Figure 2 d. Right internal carotid artery angiogram showing AVM (vertical arrow) supplied by the right ACA and an aneurysm (horizontal arrow) locating at the branch of right ACA. The aneurysm is closing to the AVM. e. Five days after GKS, the CT scan revealing a new bilateral lateral ventricle hemorrhage. f. The enhanced magnetic resonance image showing there was no AVM and aneurysm nine months after craniotomy.

Discussion

AVM with or without aneurysm may cause intracranial hemorrhage which may bring disaster to patients and their family. The relationship of AVMs and aneurysm in anatomy is important in deciding the best treatment. The classifications of the relationship between AVMs and aneurysm may summarize as: (1). Aneurysm has no relation to AVMs. (2) Aneurysm on the feeding ateries to the AVMs, which may locate on feeding artery and be proximal or distal, or within the AVMs [3, 6, 7].

It is unclear about the etiology of AVMs associating with aneurysm. There are three main points to explain the etiology. The widely accepted theory suggests that increasing hemodynamic stress was the reason of aneurysms on the walls of the major AVM feeding artery. It is supported by the fact that more aneurysms were found on feeding arteries supplying AVMs and aneurysms regress after AVM removal or occlusion [5, 8]. But Brown, et al. [5] found aneurysms associating with low-flow AVM, indicated that the mechanism was not simply for increasing hemodynamic stress. In our study, the aneurysm formed seven years after AVM diagnosis. Both AVM and aneurysm were supplied by the right ACA. Though they were close to each other, they located in different vessels. Our case doesn't support this theory above. So we think multiple factors are involved in the pathogenesis of aneurysms associate with AVMs, which need further study.

Vymazal, et al. [8] and Kim, et al. [4] reported that intranidal aneurysm or aneurysm located in the feeding artery proximal to the AVM had been cured successfully. In these two studies there was no rebleeding after the GKS. But in our study, new hemorrhage occurred in the ventricle system after GKS treatment of the AVM and aneurysm. And it is difficult to find out the main lesion causing the hemorrhage. Kano, et al. [9] reported only three of six patients associate with AVMs and aneurysm were suffered from rebleeding after stereotactic radiosurgery (SRS) caused by AVMs, while the others was unclear. In our study, DSA and enhanced MRI show that the AVM was below and close to the aneurysm, but they were located in different vessels. So we think that the GKS is not suitable for the treatment of aneurysm and AVM located in different vessels though they were close to each other.

Aneurysms, deep or periventricular location and a hemorrhage history were risk factors increasing the bleeding of AVMs after the SRS treatment [3, 10, 11]. The risk of hemorrhage after SRS is 5 times higher than without an aneurysm. However if the aneurysm is well managed, the risk of hemorrhage after SRS may reduce [9].

Conclusions

We think that the GKS is not suitable for the treatment of aneurysm and AVM located in different vessels though they are close to each other. In order to reduce bleeding again, management of the aneurysm is the best choice before GKS.

References

1. *Batjer H, Suss RA, Samson D. Intracranial arteriovenous malformations associated with aneurysms. Neurosurgery, 1986; 18: 29-35.*

2. *Thompson RC, Steinberg GK, Levy RP, Marks MP. The management of patients with arteriovenous malformations and associated intracranial aneurysms. Neurosurgery, 1998; 43: 202-211.*

3. *Redekop G, TerBrugge K, Montanera W, Willinsky R. Arterial aneurysms associated with cerebral arteriovenous malformations: classification, incidence, and risk of hemorrhage. J Neurosurg, 1998; 89: 539–546.*

4. *Kim M, Pyo S, Jeong Y, Lee S, Jung Y, Jeong H. Gamma Knife surgery for intracranial aneurysms associated with arteriovenous malformations. J Neurosurg, 2006; (Suppl) 105: 229–234.*

5. *Brown Jr RD, Wiebers DO, Forbes GS. Unruptured intracranial aneurysms and arteriovenous malformations: frequency of intracranial hemorrhage and relationship of lesions. J Neurosurg, 1990; 73: 859-863.*

6. *Cunha e Sa MJ, Stein BM, Solomon RA, McCormick PC. The treatment of associated intracranial aneurysms and arteriovenous malformations. J Neurosurg, 1992; 77: 853-859.*

7. *Perata HJ, Tomsick TA, Tew Jr JM. Feeding artery pedicle aneurysms: association with parenchymal hemorrhage and arteriovenous malformation in the brain. J Neurosurg, 1994; 80: 631-834.*

8. *Vymazal J, Novotný J, Janoušková L, Vladyka V. The role of Gamma Knife radiosurgery in arteriovenous malformation with aneurysms. Stereotactic and Functional Neurosurgery, 1999; 72: 175-184.*

9. *Kano H, Kondziolka D, Flickinger JC, Yang H, Park KJ, Flannery TJ, Liu X, Niranjan A, Lunsford D. Aneurysms Increase the Risk of Rebleeding After Stereotactic Radiosurgery for Hemorrhagic Arteriovenous Malformations. Stroke, 2012; 43: 2586-2591.*

10. *Parkhutik V, Lago A, Tembl JI, Vázquez JF, Aparici F, Mainar E, Vazquez V. Postradiosurgery Hemorrhage Rates of Arteriovenous Malformations of the Brain Influencing Factors and Evolution With Time. Stroke, 2012; 43: 1247-1252.*

11. *Kano H, Lunsford LD, Flickinger JC, Yang H, Flannery TJ, Awan NR, Niranjan A, Novotny J Jr, Kondziolka D. Stereotactic radiosurgery for arteriovenous malformations, Part 1: management of Spetzler-Martin Grade I and II arteriovenous malformations. J Neurosurg, 2012; 116: 11-20.*

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Orbital metastasis of renal cell carcinoma treated with stereotactic radiosurgery: a case report

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Abstract

We describe here a rare case of orbital metastasis of renal cell carcinoma. A 68-year old Chinese female with a history of renal cell carcinoma presented with a sudden onset of eye swelling and proptosis. Routine ophthalmologic examination indicated the presence of an orbital metastasis of renal cell carcinoma. The patient was treated with Gamma knife stereotactic radiosurgery (SRS). A 2-year follow-up examination showed that the intraorbital mass remained stable, without the need for additional treatment.

Key words: renal cell carcinoma; orbit; metastasis

Introduction

Renal cell carcinoma is the most common type of kidney tumors in adults, accounting for 80% of malignant renal tumors and 2% of systemic cancers (1). Distant metastases occur in approximately 25-30% of patients, with the most common sites being the lung parenchyma (about 50-60%), bone (about 30-40%), liver (about 30-40%), and brain (about 15%) (2). Metastasis of renal cell carcinoma to the orbit is very rare clinically. Analysis of 46 patients in southern China with cancer metastatic to the orbit showed that only 2 (4.35%) had metastases to the orbit from renal cell carcinoma (3).

Usually, ocular metastasis of renal cell carcinoma is an indicator of advanced disease. Because orbital metastasis of renal cell carcinoma is an uncommon lesion, treatment outcomes are uncertain. We describe here a patient was an orbital metastasis of renal cell carcinoma who was treated with Gamma knife stereotactic radiosurgery (SRS). A 2-year follow-up showed that the intraorbital mass remained stable, without the need for additional treatment.

Case Presentation

A 68-year-old female visited the Department of Ophthalmology at Qilu Hospital of Shandong University with a complaint of eye swelling and proptosis in her left eye over the previous 2 months. Four years earlier, she had been diagnosed with renal cell carcinoma, for which she underwent a right radical nephrectomy and the removal of a metastatic lesion from the eighth thoracic spine. Two years later, she had undergone the removal of a tumor from the twelfth thoracic spine. Her best-corrected visual acuity (BCVA) was 1.0 in the right eye and 0.3 in the left eye; with intraocular pressures of 14 mmHg and 15 mmHg, respectively; and exophthalmos of 12 mm and 16 mm, respectively. The orbital distance was 90 mm. The left eye was inwardly prominent, owed 2mm when extended and was just over the midline when turned upward. Gonioscopic examination of her right eye showed no obvious abnormalities. The anterior segment of her left eye was normal for her age. Fundus examination of the left eye showed that the optic disc boundary was unclear and the foveal reflective was missing. B ultrasound of the left eye showed that a mass, about 13.6×13.6mm in size with clear borders, was located in the posterior area of the eyeball. Orbital computed tomography revealed a mass of the left eye, indicating that this lesion was a metastasis of renal cell carcinoma (Figure 1). The patient underwent left orbital mass resection under anesthesia. The intraoperative mass was crimson in color, encapsulated and closely adherent to the superior rectus. Histologically, the tumor tissue had features of metastatic renal cell carcinoma (Figure 2). The patient was therefore diagnosed with left orbital metastasis of renal cell carcinoma, for which she underwent Gamma knife stereotactic radiosurgery (SRS). A follow-up examination after 2-years showed that

the intraorbital mass remained stable, without the need for additional treatment.

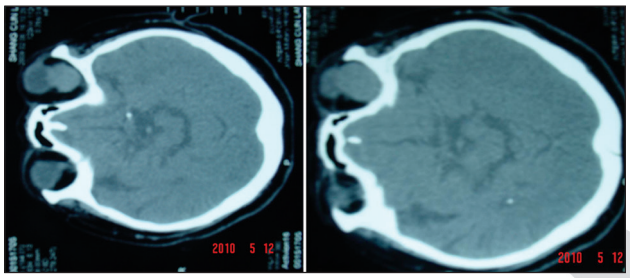

Figure 1. Representative computed tomography image of the left orbit of our patient, showing a round soft mass involving the superior and lateral rectus, compressing the optic nerve, and in close contact with the posterior wall of the eyeball

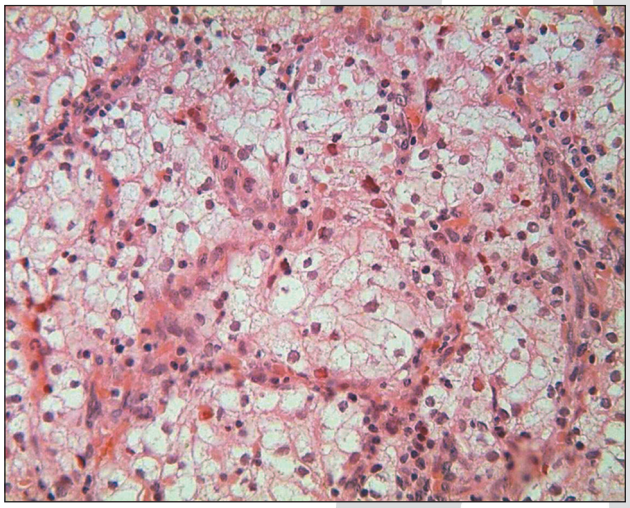

Figure 2. Intraoperative histopathological examination, showing that the tumor cells were large, round, oval or polygonal, and arranged in clusters. The cell membranes were clear with rich and transparent cytoplasm. The nuclei were round, uniformly distributed and having visible, variably-sized nucleoli. (hematoxylin and eosin staining, ×400)

Discussion

Although renal cell carcinomas frequently metastasize, ocular metastases of these tumors is very rare. The most frequent ocular metastatic site of renal cell tumors is the choroid (about 88%), followed by the iris (about 9%) and ciliary body (about 2%) (4). Metastatic emboli from the kidney are usually blood-borne, entering the eye through one or more short posterior ciliary arteries and explaining the greater frequency of metastases in the choroid than in other ocular sites.

Renal cell carcinomas have been shown to produce angiogenic factors, including fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), which can promote tumor growth and ocular neovascularization (5). The differential diagnosis of a highly vascular tumor in the eye or orbit should include metastatic renal cell carcinoma (6). One metastatic renal cell tumor presented as a pulsatile proptosis (7). In our patient, the tumor adhered closely to the surrounding tissues due to the presence in the tumor of many blood vessels.

The presence of a metastatic renal cell carcinoma in the orbit is quite uncommon. When they occur, most orbital metastases of renal cell carcinoma involve the orbital fat, muscle and bone (8). In the absence of a history of renal cell carcinoma, the diagnosis of these metastases is very difficult, usually requiring pathological evaluation.

The treatment of ocular metastases includes surgery, chemotherapy, radiotherapy and/or immunotherapy, singly or in combination. While conventional radiotherapy plays a limited and palliative role due to the relative radio-resistance of renal cell carcinoma, advances in immobilization and image guidance have led to the use of stereotactic radiosurgery (SRS) to overcome this resistance, with impressive results in the metastatic setting (9). SRS is a procedure that employs a high dose of extremely conformal radiation to treat small-sized lesions during a single treatment session. Instruments utilized to deliver SRS delivery include the linear accelerator (LINAC), CyberKnife and Gamma Knife (GK), with instrument type not affecting treatment outcomes (10). Gamma knife SRS focuses high energy, high dose irradiation on the lesion through 201 beams. An analysis of SRS in 16 consecutive patients with choroids plexus metastases, including 14 with metastases from renal cell carcinoma, found that mean survival was 25.3±23.4 months (11).

The orbital metastasis in our patient was treated successfully by Gamma knife SRS. SRS represents a safe and viable primary treatment option for these metastases, with excellent outcomes.

References

1. *Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. N Engl J Med 1996; 335(12): 865-75.*

2. *Ritchie AW, Chisholm GD. The natural history of renal carcinoma. Semin Oncol 1983; 10(4): 390-400.*

3. *Yan J, Gao S. Metastatic orbital tumors in southern China during an 18-year period. Graefes Arch Clin Exp Ophthalmol 2011; 249(9): 1387-93.*

4. *Shields JA, Shields CL, Eagle RC, Jr., Singh AD, Armstrong T. Metastatic renal cell carcinoma to the palpebral lobe of the lacrimal gland. Ophthal Plast Reconstr Surg 2001; 17(3): 191-4.*

5. *Howard GM, Jakobiec FA, Trokel SL, Iwamoto T, Jones IS. Pulsating metastatic tumor of the orbit. Am J Ophthalmol 1978; 85(6): 767-71.*

6. *Kurli M, Finger PT. The kidney, cancer, and the eye: current concepts. Surv Ophthalmol 2005; 50: 507-18.*

7. *Sharma K, Verma A, Rathi B, Kumar R, Kanaujia V. Metastatic tumor of orbit presenting as pulsatile proptosis. Ann Ophthalmol (Skokie) 2006; 38: 69-72.*

8. *Bersani TA, Costello JJ, Jr., Mango CA, Streeten BW. Benign approach to a malignant orbital tumor: metastatic renal cell carcinoma. Ophthal Plast Reconstr Surg 1994; 10(1): 42-4.*

9. *Dutcher JP, Mourad WF, Ennis RD. Integrating innovative therapeutic strategies into the management of renal cell carcinoma. Oncology (Williston Park) 2012; 26(6): 526-30, 32, 34.*

10. *Andrews DW, Scott CB, Sperduto PW, et al. Whole brain radiation therapy with or without stereotactic radiosurgery boost for patients with one to three brain metastases: phase III results of the RTOC 9508 randomised trial. Lancet 2004; 363: 1665-72.*

11. *Siomin V, Lin JL, Marko NF, et al. Stereotactic radiosurgical treatment of brain metastases to the choroid plexus. Int J Radiat Oncol Biol Phys 2011; 80: 1134-42.*

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Multiple recurrence of ectopic solitary plasmacytoma: A case report

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Introduction

Solitary plasmacytoma (SP) is an extremely rare malignant neoplasm formed of plasm cells. It contains two types: SP of bone and solitary extramedullary plasmacytoma. In this report, we describe a case of SP in the chest wall in December 2009. After surgical excision and radiotherapy 7months, SP of the right tiba was diagnosed in July 2010. A diagnosis of solitary extramedullary plasmacytoma of the adrenal was done in December 2010 after the surgery of right tiba. Reviewing of the literature, solitaty plasmacytoma was rarely to be seen. We believe the multiple ectopic recurrence of SP with unusual presentation was rarely to be seen.

Key words: Adrenal tumor, Plasmacytoma, Multiple.

Case Report

A 37-year-old male who was found to have a mass in right adrenal tumor that was revealed by ultrasonography and computed tomography (CT). The patient complained a dull epigastria pain that had persisted for 1 month. The physical examination revealed normal. Blood investigations were done which revealed Hemoglobin 148 g/L, total leukocyte count and platelet count was normal. Serum total protein, albumin, globulin and serum β2 micro globulin were normal. Examination of the endocrinologic function revealed normal levels of noradrenaline and epinephrine. Other hormonal levels were within the normal ranges. Serum IgG, IgA and IgM levels were 7.2g/L, 1670mg/L and 657mg/L, respectively. Renal function tests, serum calcium, Urinary Bence Jones proteins, Skeletal survey, Bone-marrow aspiration and biopsy were normal. The serum-Immunoelectro-Phoresis (SIEP) revealed weak M spike in gamma globulin region. On CT scans (Figure 1), a heterogeneously enhanced mass measuring 31mm×28mm×50mm was identified in the right adrenal region, adjacent to the right lobe of the liver and right kidney. No calcification was observed in this mass. The right adrenal lesion was solitary on a full-body positron emission tomography (PET). He was hospitalized as a right adrenal gland tumor, and radical resection of the right adrenal gland tumor was performed in December 2010 . During the resection, it was observed that the lesion was soft, fleshy, with a poorly defined border. The surgically excised mass (4.9cm×3.2cm) displayed a rugged surface. A hematoxylin and eosin stain of the resected sample demonstrated well-differentiated plasmacytoma consisting of mature plasmacells with diffuse distribution, there were obvious cells with mitotic figures and nucleoli (Figure 2A). Low power microscopy illustrates adrenal were infiltrated by the tumor (Figure 2B). The mass was confirmed by a histopathology, which was an extramedullary plasmacytoma.

The medial history of the patient had a SP of the left chest wall in December 2009. CT confirmed a single left chest wall tumor containing an osteolytic lesion. The lesion of the chest wall and surrounding connective tissue was excised completely. Monoclonal proliferative of Solitary plasmacytoma bone (SPB) was suggested by the histopathology, which revealed that the destructed bone marrow was substituted with abundant mature neoplastic plasma cells. A postoperative PET did not detect any distant metastasis or other bone lesions. Bone marrow biopsy was normal, and low levels of serum or monoclonal paraprotein (M-Protein) by immunoelectrophoresis. Surgical excision and radiotherapy were performed for treatment of the lesion. Yet 7 months after radiotherapy, the same diagnosis recurrence in the right tibia. Postoperative biopsy and microscope examination of the resected tissue proved to be plasmacytoma. No evidence of systemic disease on skeletal survey, and few than 2% plasma cells on bone marrow biopsy. And other features including anemia, hypercalcemia or renal disease that were related to the plasma cell neoplasm were excluded. The biopsy of the

tibia lesion showed a neoplasm composed of sheets of closely packed mature plasma cells, Tumor cell with homogenous amphophilic cytoplasm, well-type and some acidophilic nucleoli (Figure 3A). Low power microscopy illustrates osseous tissue were violated by the tumor (Figure 3B). Finally, a diagnosis of SP of the tibia was done.

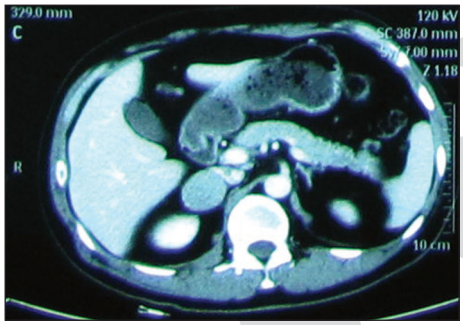

Figure 1. CT showed that a heterogeneously enhanced mass measuring 31mm×28mm×50mm was identified in the right adrenal region, adjacent to the right lobe of the liver and right kidney

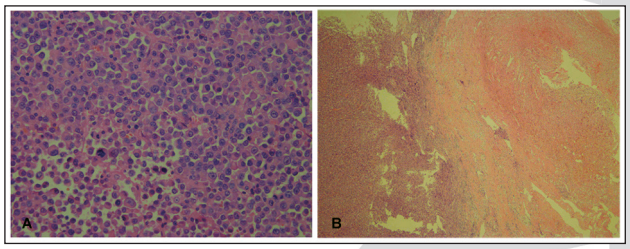

Figure 2A. Histopathology of the resected adrenal gland tumor demonstrated well-differentiated plasmacytoma consisting of mature plasmacells with diffuse distribution, there were obvious cells with mitotic figures and nucleoli (H&E, ×400). B. Low power microscopy illustrates adrenal were infiltrated by the tumor (H&E, ×100)

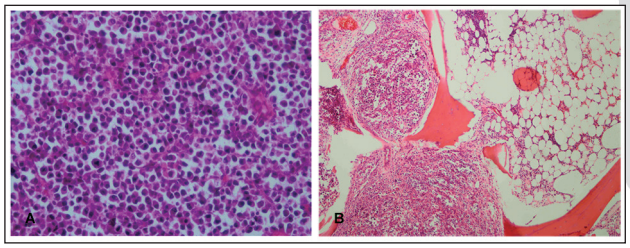

Figure 3 A. The biopsy of the tibia lesion showed a neoplasm composed of sheets of closely packed mature plasma cells, Tumor cell with homogenous amphophilic cytoplasm, well-type and some acidophilic nucleoli (H&E, ×400). B. Low power microscopy illustrates osseous tissue were violated by the tumor (H&E, ×100)

Discussion

Plasma cell neoplasms account for approximately 1% to 2% of human malignancies and occur at a rate of about 3.5/100,000 per year [1], Less than 10% of patients with plasma cell neoplasms present with a SP that may be either osseous or extramedullary. SP is an extremely rare malignant neoplasm formed of plasmacells. It divided into two categories: SP of bone and solitary extramedullary plasmacytoma.

SPB is a rare clinical entity, arising from malignant proliferation of B-cells, which accounts for approximately 3% to 5% of all plasma cell malignancies[2]. Historically, the diagnostic criteria were as follows: a solitary lesion of bone due to biopsy proven clonal plasma cells; bone marrow not consistent with multiple myeloma; normal skeletal survey; and no anemia, hypercalcemia, or renal impairment attributable to plasma cell disorder [3]. SBP is characterized by the presence of a single bone lesion, it is more common in males and median age at diagnosis is around 55years. The most common sites are bones with active bone marrow hematopoiesis such as vertebrae, ribs, skull, pelvis, femur, clavicles and scapula. Before 40 years of age, SBP is very rarely reported [4]. However, we describe a 37-year-old male, SP that was primarily found in a left wall of thorax. 7 months after radiotherapy, right tibia nearly section local recurrence was observed.

Solitary extramedullary plasmacytoma (SEP) is a plasma cell tumor arising outside of the bone marrow. SEP is an uncommon neoplasm and rarely occurs in adrenal gland. SEP accounts for approximately 3% of all plasma cell neoplasms, results from uncontrolled plasma cell proliferation and consists of monoclonal plasmacytic infiltration without bone marrow involvement[5]. The diagnosis of SEP should normally provoke investigation for disseminated disease and this should include a skeletal survey, serum and urinary protein electrophoresis, serum immunoglobulins and bone marrow biopsy[6]. SEP are highly radiosensitive, and they often respond to radiotherapy with complete clearance, Based on the documented radiation sensitivity of plasma cell tumors, the accepted treatment is radiotherapy but when a lesion can be completely resected, surgery pro-

vides the same results as radiotherapy. Combined treatment is an accepted treatment depending on the resectability of the lesion. The optimal dose for local control is 40-50 GY delivered over 4-6 weeks[7]. Adjuvant radiotherapy should be recommended to patients with positive surgical margins. The 10-year overall survival rate is 70%[8]. We describe the case of solitary extramedullary plasmacytoma of the right adrenal gland. Reports of such tumors are extremely rare.

Postoperative recovery was uneventful. Our patient did receive radiotherapy after underwent right adrenalectomy, and remained asymptomatic when we wrote this paper. Following review of the literature, we believe that this case may be the only case with the unusual presentation. This case is noteworthy, not only because of the rarity of report of SP, but also because that it is a multiple ectopic recurrence of SP.

References

1. *Bolek TW, Marcus RB, Mendenhall NP: Solitary plasmacytoma of bone and soft tissue. Int J Radiat Oncol Biol Phys, 1996; 36: 329-333*

2. *Soutar R, lucraft H, Jackson G, Reece A, Bird J, Low E, et al. Guidelines on the diagnosis and management of solitary plasmacytoma of bone and solitary extramedullary plasmacytoma.Br J Haematol 2004; 124: 717-26*

3. *Kyle RA, Child JA, Anderson K. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: A report of the International Myeloma Working Group. Br J Haematol 2003; 121: 749-757*

4. *Dumesnilc, Schneider P, Dolgopolov I, Radi S, Le Luyer B, Vannier JP. Solitary bone plasmacytoma of the spine in an adolescent. Pediatr Blood Cancer 2006; 47: 335-8*

5. *Dimopoulous MA, Kiamouris C, Moulopoulos LA. Solitary plasmacytoma of bone and tetremedually plasmacytoma. Hematol oncol clin North AM 1999; 13: 1249-1257*

6. *Tsang RW, Gospodarowicz MK, Pintile M, Bezjak A, Wells W, Hodgson DC et al: Solitary plasmacytoma treated with radiotherapy: Impact of tumor size on outcome. Int J Radit Oncol Biol Phys 2001; 50: 113-120,*

7. *Libross RH, Ha CS, Cox JD, Weber D, Delasalle K, Alexanian R et al. Clinical course of solitary extramedullary plasmacytoma. Radiother Oncol 1999; 52(3): 245*

8. *Soutar R, Lucraft H, Jackson G, Reece A, Bird J, Low E et al. Guidelines on the diagnosis and management of solitary plasmacytoma. Clin Oncol (R Coll Radio) 2004; 16(6): 405-413.*

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Late-onset small bowel obstruction following blunt abdominal trauma and mesenteric vessel damage: Case report

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Abstract

We describe a rare case in which blunt abdominal trauma resulted in mesenteric injury with delayed ischemic ileal stenosis. A gastrointestinal tract study using iodine solution demonstrated a stenotic ileal loop. Laparotomy revealed distal ileal stenosis with vascular mesenteric injury. The abnormal ileal loop was resected. Histological examination of the resected segment showed focal mucosal erosion, hyperemia, ulceration, and fibrosis of the submucosal and muscularis layers. Early diagnosis and surgical treatment can considerably reduce mortality and morbidity in these patients.

Key words: Blunt abdominal trauma, Late-onset small-bowel stenosis, Mesenteric vessel injury.

Introduction

Blunt small bowel injury is rarely diagnosed despite the fact that direct injury to the small bowel is common after blunt abdominal trauma. Much more rarely, patients can present with delayed small intestinal obstruction secondary to ischemic stricture. The interval between the initial insult to the abdomen and the obstructive episode varies widely among different reports, ranging from a minimum of 13 days [1] to a maximum of 26 years [2]. Because only a few anecdotal case reports of this complication have been reported, its precise incidence and mortality rate are unknown.

The pathogenetic mechanism by which blunt abdominal trauma might cause late bowel obstruction have not been established. The exact pathophysiology is unclear, with three possible causes cited-subclinical small bowel perforation, localized bowel ischaemia, and mesenteric vascular injury [3]. However, most reports to date have implicated mesenteric vascular injury as the cause of stricture formation [1,3,4]. According to this hypothesis, impairment of the blood supply to the bowel following mesenteric vascular damage causes hemorrhagic infarction of the mucosa, which ulcerates and subsequently heals by fibrosis and cicatricial stenosis [1].

We report here the case of a patient who presented to our hospital with small bowel obstruction 5 weeks after blunt abdominal trauma.

Case report

A previously healthy 37-year-old female was involved in a motor vehicle accident in which she suffered blunt abdominal trauma. When she was initially treated at another hospital, ultrasonography of the abdomen and pelvis revealed splenic rupture and internal hemorrhage. There was minimal fluid in the peritoneal cavity. The liver and kidneys were normal. An abdominal X-ray revealed no free air. Because she was hemodynamically stable, she was treated conservatively and discharged 1 week after admission.

Four weeks after discharge she began to experience nausea, vomiting, and abdominal pain and distension, and she presented to our emergency department. She had no history of these symptoms prior to her abdominal injury. An upright radiogram of the abdomen showed several dilated loops of small bowel containing air–fluid levels. The patient was treated with intravenous fluids and nasogastric suction, which improved her clinical status, and she was discharged 6 days after admission.

Over the next 3 months the patient continued to suffer abdominal pain. She also developed anorexia, only being able to take in small amounts of fluid, and lost 8 kg in weight. She was readmitted to our hospital for further evaluation. Gastroscopy and co-

lonoscopy were each normal. A plain radiograph of the abdomen showed small bowel distension and the presence of air-fluid levels, consistent with partial intestinal obstruction (Figure 1). In view of the potential risks of barium meal examination, we chose to carry out a gastrointestinal tract study with iodine solution. This revealed a stenotic ileal loop (Figure 2).

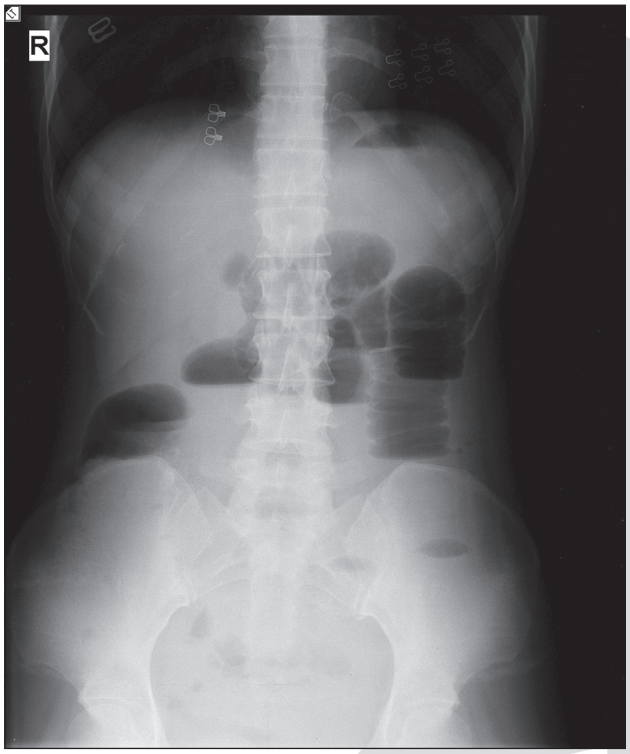

Figure 1. Plain radiograph of the abdomen showing small bowel distension and air-fluid levels

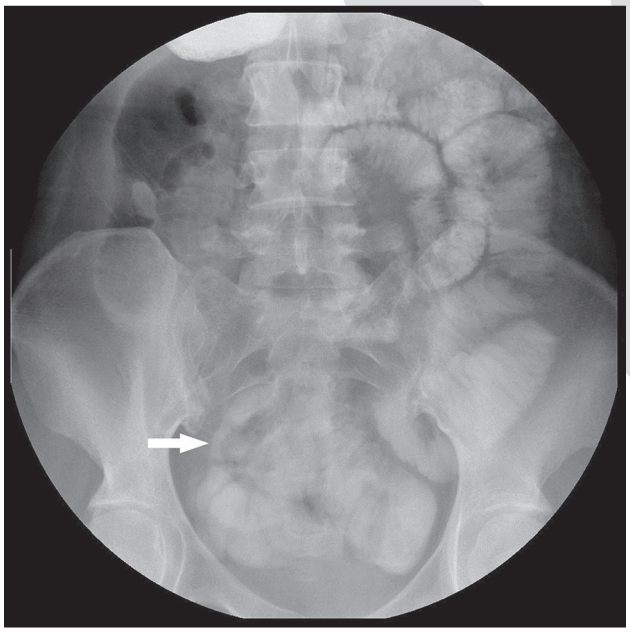

Figure 2. Abdominal iodine contrast study showing a stenotic ileal loop (arrow)

The patient underwent a laparotomy. Sixty centimeters from the ileocecal valve, we discovered a 12-centimeter-long stenotic segment of ileum, with dilation of the proximal small bowel and thickening and fibrosis of the adjacent mesentery (Figure 3). The stenotic segment of ileum was resected and a primary anastomosis was performed.

Figure 3. Photograph of the resected ileum, showing both a stenotic area and a dilated proximal segment

Examination of the pathology specimen showed focal mucosal erosion, hyperemia, ulceration, and fibrosis of the submucosal and muscularis layers. Inflammatory cells, including lymphocytes, plasmocytes, and a large number of neutrophilic granulocytes, were found to have infiltrated throughout the thickness of the bowel wall (Figure 4).

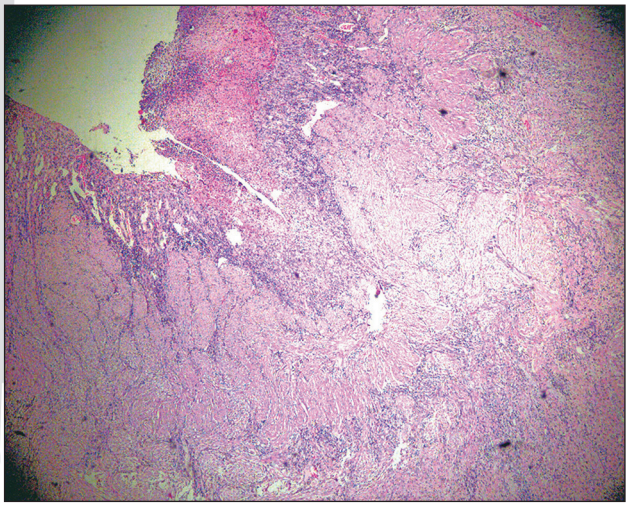

Figure 4. Photomicrograph of a section of the resected small bowel, showing mucosal erosion, hyperemia, ulceration, and fibrosis of the submucosal and muscularis layers

The postoperative course was uneventful and patient was discharged 12 days after surgery. Follow-up at 11 months found the patient to be

doing well on a normal diet, and she reported no recurrence of abdominal pain or distension.

Discussion

A number of hypotheses have been offered regarding the pathogenetic mechanism linking blunt abdominal trauma and delayed intestinal obstruction. In the current case, laparotomy revealed that the mesentery adjacent to the stenotic ileal loop was markedly thickened and fibrotic. Palpation confirmed that the vascularity of the region was impaired. Our findings thus support the most commonly proposed theory of posttraumatic small bowel obstruction, in which damage to mesenteric vessels is believed to play a critical role in triggering later intestinal stenosis.

Posttraumatic small bowel obstruction is often difficult to diagnose. In the absence of gastrointestinal symptoms and peritoneal signs, the diagnosis is often delayed while patients are treated conservatively. Some authors believe that a small bowel barium swallow study is the best technique for demonstrating lesions of the small intestine, as it most clearly shows the contours of the bowel lumen and the location of the stricture [1,5]. However, the use of barium in patients with suspected small bowel obstruction not only increases the risk of worsening obstruction, but it can also interfere with computed tomography studies. In the current case, the patient had severe obstructive symptoms. An examination using iodic water clearly demonstrated a stenotic ileal loop, which aided in both preoperative diagnosis and treatment.

If possible, CT should be the first examination made in suspected cases of post-traumatic small bowel stricture, as it can identify reliably most significant bowel and mesenteric injuries [4,6]. In cases of small bowel obstruction secondary to delayed stricture formation, abdominal CT will often show an ileal loop with a thickened wall and a narrowed lumen [4,6-7]. However, it should also be taken into consideration that CT scan has a high false-negative rate, which may delay the diagnosis [8].

Intestinal stricture causing small bowel obstruction after blunt abdominal trauma is rare, and thus may not be high on the diagnostician's differential list. Because the disease is irreversible with conservative treatment, the only reliable treatment is surgical resection of the stenotic small bowel loop and primary anastomosis. A high index of suspicion is appropriate when any patient presents with symptoms of bowel obstruction weeks or years after sustaining abdominal injury, and in these cases abdominal CT and contrast studies should be performed. Early diagnosis and surgical treatment can considerably reduce mortality and morbidity in these patients.

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References

1. *Bryner UM, Jerrolo K. Post-traumatic ischaemic stenosis of the small bowel. Arch Surg. 1980; 115: 1035-41.*
2. *Vanderschot PM, Broos PL, Gruwez JA. Stenosis of the small bowel after blunt abdominal trauma. Unfallchirurg. 1992; 95: 71-3.*
3. *Maharaj D, Perry A, Ramdass M, Naraynsingh V. Late small bowel obstruction after blunt abdominal trauma. Postgrad Med J. 2003; 79: 57-8.*
4. *Bougard V, Avisse C, Patey M, Germain D, Levy-chazal N, Delattre JF. Double ischemic ileal stenosis secondary to mesenteric injury after blunt abdominal trauma. World J Gastroenterol. 2008; 14: 143-5.*
5. *Hirota C, Iida M, Aoyagi K, Matsumoto T, Yao T, Fujishima M. Post-traumatic intestinal stenosis: clinical and radiographic features in four patients. Radiology. 1995; 194: 813-5.*
6. *Bala M, Lebenthal A, Pikarsky E, Faroja M, Rivkind AI, Mintz Y. Intestinal stenosis causing small bowel obstruction after nonoperative management of blunt abdominal trauma. J Trauma. 2007; 62: 1511-3.*
7. *Tsushima Y, Yamada S, Aoki J, Endo K. Ischaemic ileal stenosis following blunt abdominal trauma and demonstrated by CT. Br J Radiol. 2001; 74: 277-9.*
8. *Goffette PP, Laterre PF. Traumatic injuries: imaging and intervention in posttraumatic complications (delayed intervention). Eur Radiol. 2002; 12: 994-1021.*

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Ruptured adrenal pheochromocytoma presented as massive hematoma: A case report and literature review

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Abstract

The adrenal Pheochromocytoma is extraordinary tricky tumor with extremely various and unexpectedly complicated manifestations, which often delayed the management of some patients due to no timely and correct diagnosis. The pheochromocytoma with the typical adrenergic symptoms can easily cause clinician, s vigilance and gain timely diagnosis, whereas neither the medical history nor the typical manifestations, presented as cardiovascular diseases or retroperitoneal hematoma, sometimes would result in misdiagnosis even catastrophic outcome. We described a female patient who presented as retroperitoneal massive hematoma resulted from the ruptured adrenal pheochromoctyoma, coupled with the normal level of serum catecholamine, which made it was more difficult to obtain correct diagnosis. Consequently, the laparotomy was performed for discovering the factors. The pathological evaluation confirmed the diagnosis. So we had been reviewed relative literatures and stated our experience so as to raise the vigilance of clinicians and avoid awkward consequence.

Key words: Pheochromocytom, adrenal hemorrhage, retroperitoneal hematoma.

Introduction

The pheochromocytoma arose from the chromaffin cells of adrenal medulla and ganglion of sympathetic nerve, which can automatically secrete excessive catecholamine were responsible for various symptoms [1,2,3]. During the operative or postoperative phage, Some patients might occur as life-threatening hemodynamic disturbance and arrhythmia even catastrophic consequences. In the previous literature the surgical mortality rates had indicated that up to 20%～50% in the pheochromocytoma without correct diagnosis and adequate preparation[4]. The patients with spontaneous ruptured pheochromocytoma presented as retroperitoneal massive hematoma are uncommon conditions, which had been reported only approximately 50 cases in the previous literature [5,6]. We herein described a case of spontaneous ruptured adrenal pheochromocytoma, but presented as retroperitoneal massive hematoma initially. Then the patient received the surgery and the pathology confirmed the diagnosis.

Case report

A 66-year-old elderly female, without any causative factors, complained of the left back pain and nausea for 5h. She had been healthy and no the history of contagious diseases and surgery. Physical examinations demonstrated that the left low back was tenderness, no guarding and masses. Abdominal ultrasonic and computer tomography revealed that a cystic or parenchymatous mass about 11×9.1 cm2 in the left retroperitoneal space (Figure 1). Laboratorial tests demonstrated that her serum catecholamine was normal range and other studies were not significant. Thus the case underwent laparotomy and a dark black oval cystic mass about up to 11cm in diameter was found in left adrenal area, and surrounded by many expansive and circuitous blood vessels. The mass closely adhere to circumambient organs. The left kidney was compressed to downward. After ligation of the main vein of adrenal gland, the mass was mobilized and removed. The mass contained furvous blood about 500ml. The patient had not obvious blood pressure fluctuation in the course of operation and recover well. The pathology and Immunohistochemical study confirmed the diagnosis of adrenal pheochromocytoma. (as show Figures A, B). The patient has a follow up and *no recurrent and metastasis as far.*

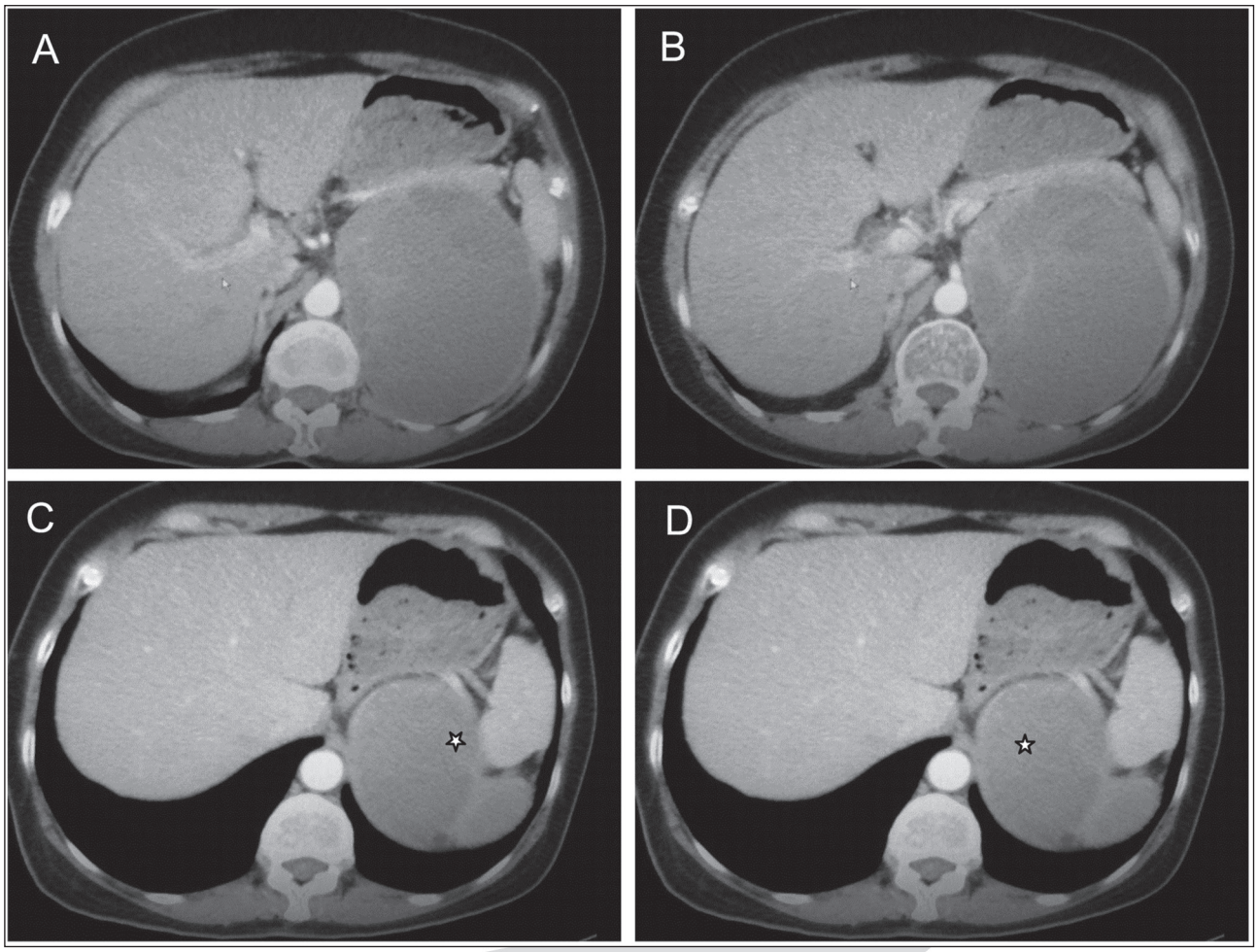

Figure 1. Enhanced abdominal computer tomogram scan showed a large (11 × 9.1cm²) cystic or parenchymatous tumor at the left adrenal gland region and mass compress left kidney, the relation between upper pole of left kidney and tumor are not distinct and the adrenal do not appear. Arrowhead indicates the tumor and asterisk indicates upper pole of left kidney

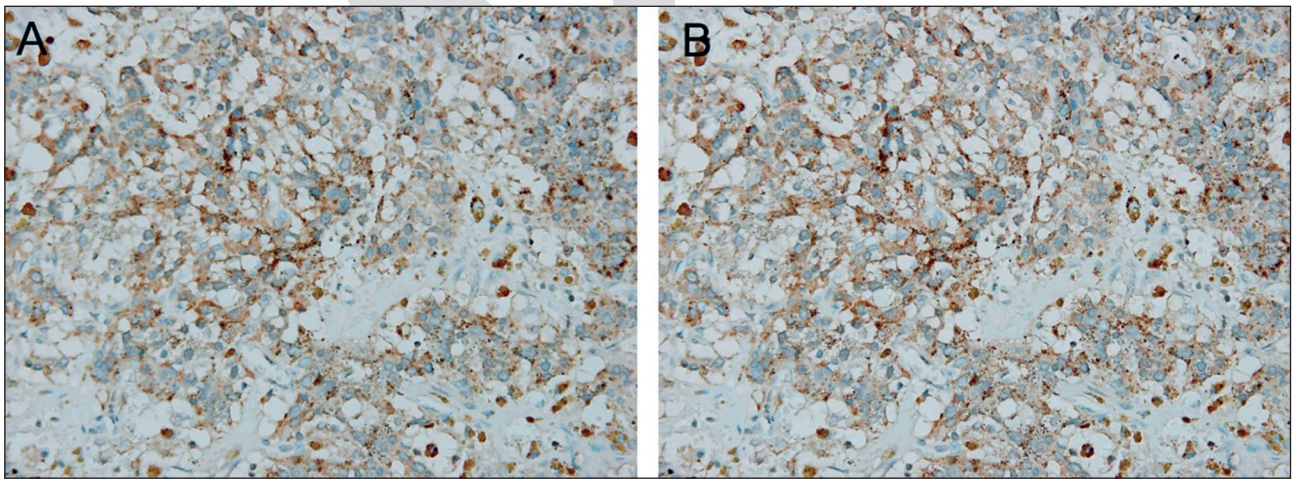

Figure 2A. Indicate CgA(+); B: indicate syn (+)

Discussion

90% of the pheochromocytoma located in the adrenal medulla and implicated unilateral adrenal gland[7]. The annual incidence is less than eight cases per million had been reported in previous literatures[8]. However, in some adrenal lesions with the surgical treatment had been demonstrated that the pheochromoctyoma were diagnosed between 18.8% with 29.9%[9,10,11], which undoubtedly suggested that the pheochromoctyoma occupied a significant share in the adrenal field.

Because of the pheochromocytoma can automatically secrete excessive catecholamine and led to the diversity of manifestation [12]. Sometimes occurs as heart failure, shock and intensive abdominal pain and even the ventricular tachycardia, acute myocardial infarction and dramatic cyclic hemodynamic fluctuations had also been documented [13,14,15,16]. Nevertheless, the cases with pheochromocytoma did not present as the symptoms were up to 10～13.9% had been documented [17,18]. Niemann at al, had described that 5%～10% of cases did not occur as hypertension, under the condition of stress state, presented as hypertension, even pulmonary or cerebral edema [4] .

With increasing development of imaging technique, ultrasonography, computed tomography and magnetic resonance imaging can all detect distinctively adrenal lesion[20,19]. ^{131}I-metaiodobenzylguanidine scintigraphy play a critical role in indicating ectopic pheochromocytoma, and positron emission tomography(PET) had begin to apply to detect the pheochromocytoma , however, the reason why difficult popular is that expensive cost[21]. In our case, the retroperitoneal hematoma was very obvious and covered the nature of adrenal neoplasia. Similarly Certain adrenal hematomas were taken for pheochromocytoma had been described in previous literatures[22,23, 24]. The patient with simple hematoma probably may avoid surgical operation through either closely observation or transcatheter arterial embolization to prohibit continuing bleed[25]. So it is significant that investigate the catecholamine of serum and urine. However, some pheochromocytoma did not appear abnormal catecholamine[11]. As our case,her blood pressure and serum catecholamine were normal limits. Therefore ours diagnosis inclined to the adrenal tumor hemorrhage and an explorative operation had been performed. Fortunately, the patient did not occur as hemodynamic disturbance in the course of operation. We had considered that the massive hematoma compressed the adrenal medulla and resulted in decreasing blood supply, so that producing and secreting catecholamine reduced. Furthermore we clipped and disconnected the vessels of circum-hematoma prior to the manipulation of hematoma, which contribute to reduce the releasing of catecholamine.

Adrenal gland spontaneous hemorrhage was an uncommon condition had been generally associated with the conditions included trauma, retroperitoneal surgery, highly stress, over-anticoagulant therapy, abnormal clotting mechanism and sepsis etc[26 ,27,28]. In previous literature the spontaneous ruptured adrenal pheochromoytoma resulted in retroperitoneal hemorrhage had also been reported[5,29]. As described in our case, no trauma and stress, spontaneous hemorrhage and developed into a large hematoma. So it was an extraordinarily rare instance.

The pheochromocytoma could secrete excess catecholamine result in sustained vasoconstriction, although the blood pressure was very high, the plasmatic volume was insufficient. So it was exceedingly dangerous to abruptly perform operation before relaxing blood vessels and expanding plasmatic volume.[30] In a survey of 15 spontaneous ruptured pheochromocytoma, had been suggested that 4 cases died from pulmonary edema in either operative or postoperative period (all 4 cases were emergent operation and 2 cases had unknown diagnosis preoperative period). So they considered that the adequate preoperative preparation was critical to reduce mortality rate. However the opposing concept argued that the patients without preoperative preparation, under the perfect monitoring and cooperation of anaesthesia, could still attain successful surgery [31], As our case shown that the patient did not use alpha-blocker or beta-blocker for the preoperative preparation, with the perfect anesthetic monitoring, the operation had still been performed successfully.

Conclusion

The Pheochromocytoma can secrete excessive catecholamine led to various manifestations, often delayed the treatment of some patients. Therefore, we suggested that the possibility of pheochromo-

cytoma should be considered under the conditions such as the recurrent hemodynamic disturbance and retroperitoneal hematoma regardless whether the serum catecholamine abnormality or not.

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References

1. *Kwon HJ, Park JH, Jin GY, Chung MJ, Lim ST, Kim MH, Kim HJ, Lee YC. Posterior mediastinal paraganglioma with bilateral adrenal pheochromocytoma. Respir Med. 2004; 98(6): 574-6.*

2. *Lenders JW, Eisenhofer G, Mannelli M, Pacak K. phenchromocytoma. Lancet. 2005 Aug 20-26; 366(9486): 665-75. Review.*

3. *Hernandez FC, Sanchez M, Alveres A, et al. A five-year report on experience in the detection of pheochromocytoma [J]. Clinical Biochemic. 2000; 33(8): 649-655.*

4. *Niemann U, Hiller W, Behrend M. 25 years experience of the surgical treatment of phaeochromocytoma [J] . Eur J Surg. 2002; 168(12): 716-719.*

5. *Arora S, Vargo S, Lupetin AR. Computed tomography appearance of spontaneous adrenal hemorrhage in a pheochromocytoma. Clinical Imaging. 2009 Jul-Aug; 33(4): 314-7.*

6. *Kobayashi T, Iwai A, Takahashi R, Ide Y, Nishizawa K, Mitsumori K. Spontaneous rupture of adrenal pheochromocytoma: review and analysis of prognostic factors. Journal Surgical Oncology, 2005; 90: 31–5.*

7. *Louafy L, Lakhloufi A, Hamdaoui R, Chehab F, Khaiz D, Bouzidi A. Non-functional retroperitoneal paraganglioma. Prog Urol. 2001 jun; 11(3): 512–6.*

8. *Sutton MG, Sheps SG, Lie JT. Prevalence of clinically unsuspected pheochromocytomas. Review of a 50-year autopsy series. Mayo Clin Proc. 1981 jun; 56: 354–60.*

9. *Chavez-Rodriguez J, Pasieka JL. Adrenal lesions assessed in the era of laparoscopic adrenalectomy: a modern day series. Am J Surg.2005 May; 189(5): 581-5; discussion 585-6.*

10. *Bin X, Qing Y, Linhui W, Li G, Yinghao S. Adrenal incidentalomas: Experience from a retrospective study in a Chinese population. Urol Oncol. 2011 May-Jun; 29(3): 270-4.*

11. *Kirshtein B, Pagliarello G, Yelle JD, Poulin EC . Incidence of pheochromocytoma in trauma patients during the management of unrelated illness: A retrospective review. Int J Surg. 2007; (5)5: 332-335.*

12. *Liao WB, Liu CF, Chiang CW, et al. Cardiovascular manifestations of pheochromocytoma [J] . Am J Emerg Med. 2000(5); 18: 622-625.*

13. *Li WM, Huang CH, Su CM, et al. Extra-adrenal pheochromocytoma presenting with life-threatening ventricular tachycardia: a case report. Kaohsiung J Med Sci. 2004; 20(12): 612-5.*

14. *Brilakis ES, Young WF Jr, Wilson JW, et al. Reversible catecholamine-induced cardiomyopathy in a heart transplant candidate without persistent or paroxysmal hypertension. J Heart Lung Transplant. 1999; 18(4): 376-80.*

15. *Chen HY. Ruptured pheochromocytoma presents as acute myocardial infarction. Am J Emerg Med. 2009; 27(8): 1018.e5-6.*

16. *Guzik P, Wykretowicz A, Wesseling IK, Wysocki H. Adrenal pheochromocytoma associated with dramatic cyclic hemodynamic fluctuations. Intl J Cardil. 2005; 103(3): 351-3.*

17. *Sutton MG, Sheps SG, Lie JT. Prevalence of clinically unsuspected pheochromocytomas. Review of a 50-year autopsy series. Mayo Clinic Proc. 1981; 56(6): 354–60.*

18. *YunChao G. The progress of the pheochromocytoma. Labeled immunoassay and clinical medicine. 2007; 14(4): 256-9.*

19. *Shen SH, Wang JH, Chen KK, Pan CC. Retroperitoneal glomus tumor: CT and MRI appearance. European Journal of Radiology Extra. 2011; 77: e79-83*

20. *Wan YL. Ultrasonography of the Adrenal Gland. J Med Ultrasound. 2007; 15(4): 213-27.*

21. *Wong KK, Arabi M, Bou-Assaly W, Maria MC , Rubelloc D, Gross MD, et al. Evaluation of incidentally discovered adrenal masses with PET and PET/CT. Eur J Radil.2012 Mar; 81(3): 441-50.*

22. *Khan A , Mutazindwa T, Hassan A, Al-Zuhair N. Unilateral traumatic adrenal haematoma presenting as pheochromocytoma. Eur J Radiol. 1998; 28(2): 133-5.*

23. *Hu XQ, Tian H. adrenal hematoma: a report of 4 cases and review of chinese literature. Med J Chin PLA . 2003; 28(5): 410-2.*

24. *Jiang YS, Jin TY ,Huang BZ, et al. A case of massive oldern adrenal hematoma. Journal of Medical Science YanBian University. 1997; 20(4): 242.*

25. *Liu TG, Han Hl, Sun JL .The clinical application of preoperative embolization before surgical treatment of adrenal pheochromocytoma. J Med Imaging. 2011; 21(1): 82-4.*

26. *Bittencourt JA, Averbeck MA, Schmitz HJ. Hemorrhagic shock due to spontaneous rupture of adrenal pheochromocytoma. Int Braz J Urol. 2003; 29: 428-30.*

27. *Sumino Y, Tasaki Y, Satoh F, Mimata H, Nomura Y. Spontaneous rupture of adrenal pheochromocytoma. J Urol 2002; 168: 188-9.*

28. *Mussig K, Horger M, Haring H, Wehrmann M. Spontaneous rupture of malignant adrenal pheochromocytoma. Emerg Med J.2008; 25(4): 242.*

29. *Wang ZL, Zhao J, Yu RL, Wang XF, Dong Z, Yang XK. Spontaneous rupture of adrenal heochromocytoma (report of 2 cases and literature review). J Modern Urol. 2004; 9(4): 230-231.*

30. *Tanaka K, Noguchi S, Shuin T, Kinoshita Y, Kubota Y, Hosaka M. Spontaneous rupture of adrenal pheochromocytoma: a case report. J Urol. 1994; 151(1): 120- 121.*

31. *Chen ZF, Qin ZK, Zhou FJ, Han H, Liu ZW, Zhang DZ, Yang JA. Emergent Treatment for Latent Pheochromocytoma Diagnosed during Operation: A Report of 11 Cases. Chinese Journal of Cancer. 2008; 27(5): 531-534.*

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Does the number of forensic cases decrease in Ramadan?

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Abstract

Objective: Fasting during the month of Ramadan in Muslim societies is of great importance for many individuals with religious, psychological and socio-cultural perspective to practice self-control. The period of Ramadan can affect both cognitive and physical functions of people. However, there is no study reported in literature about the effects of Ramadan on forensic cases. This study aims to assess the effect of Ramadan on forensic cases that come to emergency room.

Methods: We retrospectively investigated forensic cases that come to the emergency room of the Community Hospital between the dates 01.01.2008-01.10.2011. The effect on the number of forensic cases were analyzed in the months of Ramadan.

Results: During the four year period, a total of 643,488 patient came to the emergency room of which 9794 of these patients were defined as forensic cases. Out of total reported forensic cases, 995 cases (10.2%) were reported during the month of Ramadan (Chi-square, p= 0.302). The mean age of the forensic cases during the Ramadan period is 31± 14.9, however, the mean age for other months is 32.3±15.4 (t-test, p=0.01).

Conclusion: Consequently, we could not detect any effect of Ramadan on the forensic cases that come to the emergency room. This implies that, Ramadan has no effect on the types of forensic cases, but we can conclude that the Ramadan period has no effect or induce any change in daily lives of people.

Key words: Emergency room, forensic case, Ramadan.

Introduction

In general, cases which involve actions against people are called forensic cases, even if these actions do not result in injury or death by injury. In other words, events that occur due to external factors and that cause deterioration of people's physical and mental health or their death are considered forensic cases (1). Assaults, stab injuries, sexual assaults, murder, miscarriage, traffic accidents, home accidents, occupational accidents, burns, electrical injuries, fall from height, drug-substance-gas intoxications, suicide attempt, disability and death due to negligence are examples of forensic cases. Many of these cases are detected at emergency departments.

During Ramadan, approximately one billion Muslims avoid food and drink between sunrise and sunset, and they generally eat full meals after sunset and before sunrise. Fasting time changes from 13 to 18 hours. Ramadan fasting is one of the most admired rituals in Islamic culture and unhealthy and weak individuals are exempted from fasting. Many people fast periodically due to health, religious or cultural motivations (2,3).

Ramadan affects an important portion of the world population and there are studies related to its health and social impacts (4-7). People make some arrangements in their lives as required by the Ramadan ritual. They avoid activities such as using alcohol, substance, drugs, night life etc. The aim of this study is to investigate the effect of Ramadan on the number of forensic cases that present to the emergency department.

In our search for studies on forensic cases, although we found studies on forensic cases that present to the emergency department, we did not came across previous studies with respect to the Ramadan period.

Material and Method

This study was conducted between 01.01.2008 and 01.10.2011 as a retrospective data evaluation of the records of patients that presented to the Community hospital emergency department. Among these, patients who were considered forensic cases (traffic accident, stab injury, fall from height, occupational accident, suicide, assault, alcohol and drug use, firearm injury, etc.) were divided into two groups with respect to their admission dates; Ramadan and Non-Ramadan periods. For 2008, September 1st - September 30th; for 2009, August 21st - September 20th; for 2010, August 11st - September 9th and for 2011, August 1st - August 30th were defined as official periods for Ramadan. One month before and one month after Ramadan were defined as Around-Ramadan periods.

Forensic cases that occurred during the study period were examined. Within this examination, demographic characteristics such as age and gender, type of the forensic case (traffic accident, sharp object injury, fall, occupational accident, suicide, assault, alcohol and drug use, firearm injury), admission date (Ramadan, Around-Ramadan and other periods) and final state of the patient were evaluated. Forensic cases that lacked information in patients' data files were excluded from the study. 11,234 forensic case files were found for this period. 9,794 of these cases which had complete data files were included in the study.

Study data was recorded in the SPSS 16.0 software. The number of patients who presented to the hospital and considered forensic cases were compared for Ramadan, Around-Ramadan and other periods. Chi-square test was used in the data analysis.

Results

During the period of 4 years for which the study was carried out, a total number of 643.488 patients were found to have presented to the emergency department. 9,794 of these patients (1.5%) were considered forensic cases. 3,214 (32.8%) of these forensic cases were female and 6,580 (67.2%) of them were male.

Average age of the forensic cases was calculated as 32.1±15.3. Average age for females was calculated as 32.3±16.6 and average age for males was 32.1±14.7 (t-test, p=0.563).

3,426 (35.0%) forensic cases during the study period were incidents that arrived to the emergency department due to traffic accidents. Distribution of forensic cases is shown in Table 1.

Exitus occurred in 38 (0.4%) forensic cases that were included in the study. Final states of the forensic cases are shown in Table 2.

Distribution of the forensic cases that were included in the study over years is shown in Table 3.

995 forensic cases were found to have presented in Ramadan during the study period.

Forensic cases in Ramadan and Non-Ramadan periods are shown at Table 4 with respect to years.

Forensic cases presented in Ramadan and Around-Ramadan periods are shown in Table 5 with respect to years.

Throughout the study period, while average age for forensic cases presented in Ramadan was 31.0±14.9, the average age was 32.3±15.4 for Non-Ramadan (t-test, p=0.010).

While 305 forensic cases in Ramadan were female patients, 2,909 of forensic cases were female for Non-Ramadan period (chi-square, p=0.125).

Table 1. Types of forensic cases

Types of Forensic Cases	Number of Forensic Cases	Percentage (%)
Trafik kazası traffic accidents	3426	35,0
Occupational accidents	700	7,1
Suicide	1393	14,2
Firearm injuries	118	1,2
Stab injuries	769	7,9
Downfalls	523	5,3
Assaults	2001	20,4
Alcohol+Drugs	81	,8
Other	538	5,5
Fall from height	245	2,5
Total	**9794**	**100,0**

Table 2. Final states of forensic cases at the emergency department

Final State	Number of Forensic Cases	Percentage (%)
Outpatients	8054	82,2
Hospitalized patients	918	9,4
Lay in the intensive care unit	163	1,7
Referred to other hospital	100	1,0
Exitus	38	,4
Unknown	352	3,6
Refused treatment	169	1,7
Total	**9794**	**100,0**

Table 3. Distribution of forensic cases over years

Year	Number of Forensic Cases	Percentage (%)
2008	2315	23,6
2009	2039	20,8
2010	2989	30,5
2011	2451	25,0
Total	**9794**	**100,0**

Table 4. Distribution of forensic cases by year (Ramadan / Non-Ramadan)

Year	Forensic cases in Ramadan / Non-forensic cases in Ramadan	Forensic cases (Except Ramadan) / Non-forensic cases (Except Ramadan)	Number of total forensic cases in Emergency Deparment / Number of total patients who admitted to Emergency Department	P value*
2008	235 / 14.265	2080 / 148343	2315 / 164.923	0.020
2009	208 / 14.802	1831 / 152068	2039 / 168.909	0.036
2010	337 / 16.759	2652 / 150393	2989 / 170.141	0.025
2011	215 / 16.541	2236 / 120523	2451 / 139.515	<0.001
Total	995 / 62.397	8799 / 633694	9794 / 643.488	0.302

**Chi-square test*

Table 5. The number of forensic cases by year (Ramadan / Around-Ramadan)

Year	Forensic cases in Ramadan / Number of non-forensic cases in Ramadan	Forensic cases around Ramadan (2 Months) / Non-forensic cases around Ramadan (2 Months)	P value*
2008	235 / 14.265	507 / 30.787	0.996
2009	208 / 14.802	466 / 29.396	0.151
2010	337 / 16.759	689 / 33.141	0.619
2011	215 / 16.541	667 / 34.510	<0.001
Total	995 / 62.397	2329 / 127.834	<0.001

**Chi-square test*

Table 6. Distribution of forensic case types in Ramadan

	Forensic cases in Ramadan (4 years)	Forensic cases in Non-Ramadan (4 years)	P value*
Traffic accidents	371	3055	0.108
Assaults	208	1793	0.696
Suicide	137	1256	0.665

**Chi-square test*

Distribution analysis of the most frequent forensic cases in Ramadan and Non-Ramadan are shown in Table 6.

987 of 2,227 female forensic cases were presented to the emergency department due to suicide attempts where 406 of 6,174 male cases were due to the same cause (chi-square, $p<0.001$). 70.9% of the forensic cases presented to the emergency department due to suicide attempts were female, 29.1% of them were male.

32.7% of the forensic cases presented to the emergency department due to traffic accidents were female, 67.3% of them were male. 25% of the cases due to assaults were female, 75% of them were male.

With respect to final states of forensic cases, 853 (85.7%) forensic cases in Ramadan and 7,201 (81.8%) cases in Non-Ramadan were discharged (chi-square, $p=0.002$).

Discussion

According to the results of the research, 995 people presented to the emergency department as forensic cases in Ramadan periods during the study and this number formed 10.2% of the total forensic cases. An examination of the forensic cases by year show that the number of forensic cases in emergency department increased in 2010 with respect to other years. In literature, it has been reported that 5.3% of patients who presented during the three-year period were forensic cases (8). In our research, this rate is observed to be 1.5% according to our hospital's records. The fact that there are four major hospitals (two university and two training and research hospitals) around the hospital where the study was conducted might have effected patient distribution. Furthermore, traffic accidents that form the majority of the forensic cases are distributed to the hospitals by the 112 emergency service. The fact that the rate of patient transfer by 112 emergency ambulances to our hospital is low compared to the other hospitals may be a reason this.

In this study, 2008–2011 Ramadan periods coincided with summer months. An analysis by year showed that there was a statistically significant increase in forensic cases for each year's Ramadan period ($p<0.05$). However, after a four-year overall evaluation, no statistically significant increase was observed in forensic cases ($p=0.302$). This paradox can be explained by the effect of the increase in the number of forensic cases in summer months as stated in the above literature. And this shows clearly that Ramadan period has no real effect on forensic cases detected at emergency departments. As a supporting observation, evaluation by year indicates that there is no statistically significant difference between Ramadan and Around-Ramadan periods (Table 6).

A high discharge rate (82.2%) was found in Ramadan period among final states of patients considered forensic cases. Examination of final states of forensic case patients shows that intensive care, hospitalization and treatment rejection rates are higher for suicide cases compared to other cases. Moreover, there was no forensic case resulting in death in Ramadan. Although Ramadan has physiological and psychological effects on a fasting person, it seems to have no positive effect in the final states of forensic cases in Ramadan period. One might think that lower hospitalization rate of these cases may be imposing a lower effect of these incidents.

A previous study reports that approximately 68% of the forensic cases were male. The fact that similar studies also indicate higher rates of males in forensic cases shows that males participate in social life more than females and hence they carry a higher risk of being exposed to traumas (1,9,10). An analysis of forensic cases by gender shows that female cases that presented to the emergency department due to suicide attempt are more than male cases (chi-square, $p<0.001$). As stated in Turkish Statistical Institute's suicide statistics, between 2001-2010, the highest rate of suicide was observed in 15-24 age group and the majority of them were women.

Similarly, Z. Çakir et al. in their study report that the average age was 27.8±17.8 and state that male gender was clearly at a higher rate (11). In this study, 67% of the forensic cases belonged to males and the average age was 32.1 where gender was not taken into account. Türkmen et al. indicate the average age of forensic cases as 28.7 in their study (1). Literature suggests that trauma cases are the primary causes for deaths occurring between 1-44 ages and that they form the major part of the forensic cases. Also in the study by H. Akoğlu et al., 33% of trauma patients were female, 67%of them were male (12).

In this study we observed that the average age of forensic cases presented in Ramadan is lower than the average in Non-Ramadan period (t-test, p=0.01). However, no statistically significant difference was observed when compared to one-month Around-Ramadan periods (t-test, p=0.268). This fact might suggest that difference in age is not due to effect of Ramadan but it can be a result of seasonal influence. During the study Ramadan months coincided with summer season.

Research on seasonal influence on suicide cases showed that there were more suicide cases in the summer (13, 14). People with psychological disorders attempt suicides generally in summer. It has been stated that suicidal tendency has a positive correlation with the rise in temperature and sunlight intensity while it has a negative correlation with cloudy weather and high barometric pressure.

The reason why males are involved in more forensic cases may be that there are more male drivers and that violent incidents such as assault and physical injuries are more common between them. Altun et al. state that 85% of the assaults in their study were between males (15). Assaults are frequently observed in secondary and high school male students. This suggests that the increase in tendency to fight and violence at these ages might be a reason (15, 16). The majority of the traffic accident injuries in Turkey involve young-male population. It was found that young men and old women drivers carry the highest risk of getting involved in a traffic accident (17).

It was found that after traffic accidents, most frequent reasons for admissions are intoxication and suicide attempts, assault and fall from height (15,16).

We observed that traffic accidents are the primary causes of forensic cases in both Ramadan and Non-Ramadan periods (chi-square, p=0.108). In United Arab Emirates Bener A. et al. found that traffic accident rate is highest during Ramadan. Traffic accidents form the majority of the forensic cases independent of Ramadan period (18).

Al Suwaidi J. et al. reported that external factors related to Ramadan; changes in food intake and/or sleeping times can affect circadian rhythm (19). Again in other studies, it is shown that sudden change in meal times effects sleeping times and circadian rhythm (5). Night sleep, day alertness and psychomotor performance decrease (6). Although Ramadan's effect on individual performance in literature suggests that there might be an increase in traffic accidents, sharp object injuries and fall cases; in this study it was found that Ramadan has no statistically significant effect on this distribution (p=<0.05).

Conclusion

It was observed that there is no significant difference between forensic cases presented in Ramadan and Non-Ramadan periods. No difference compared to common literature was found in gender distribution of forensic cases in Ramadan. Average age of forensic cases in Ramadan was lower than to that in Non-Ramadan period. Forensic cases in Ramadan had a higher rate of discharge compared to Non-Ramadan forensic cases.

References

1. *Türkmen N, Akgöz S, Çoltu A, Ergin N. Evaluation of Legal Cases Admitted to Uludağ University Medical School Emergency Department. Uludağ Medical Journal 2005; 31(1): 25-29.*

2. *Zare A, Hajhashemi M, Hassan ZM, Zarrin S, Pourpak Z, Moin M et al. Effect of Ramadan fasting on serum heat shock protein 70 and serum lipid profile. Singapore Med J 2011 Jul; 52(7): 491-5.*

3. *Maughan RJ, Fallah J, Coyle EF. The effects of fasting on metabolism and performance. Br J Sports Med 2010 Jun; 44(7): 490-4.*

4. *Azizi F. Islamic fasting and health. Ann Nutr Metab 2010; 56: 273–282.*

5. *BaHammam A, Alrajeh M, Albabtain M, Bahammam S, Sharif M. Circadian pattern of sleep, energy expenditure, and body temperature of young healthy men during the intermittent fasting of Ramadan. Appetite 2010 Apr; 54(2): 426-9.*

6. *Roky R, Houti I, Moussamih S, Qotbi S, Aadil N. Physiological and chronobiological changes during Ramadan intermittent fasting. Ann Nutr Metab 2004; 48(4): 296-303.*

7. *Topaçoğlu H, Karcioğlu Ö, Yürüktümen A, Kiran S, Cimrin AH, Ozucelik DN et al. İmpact of Ramadan on demographics and frequencies of disease-related visits in the emergency department. Int J Clin Pract 2005 Aug; 59(8): 900-5*

8. *Yavuz MS, Özgüner İ. Süleyman Demirel Üniversitesi Tip Fakültesi Acil Servisi'ne müracaat eden adli olguların değerlendirilmesi. Turkish Journal of Forensic Medicine 2003; 17(1): 47-53.*

9. *Küçüker H. Evaluation of Nonlethal Traumatic Forensic Cases and Reports Who Admitted To Emergency Services. Tr J Emerg Med 2003; 3(4): 19-23.*

10. *Canbaz K. İlk ve Acil Yardim Basamağinda Adli Olgularin Değerlendirilmesi. İstanbul Üniversitesi İstanbul TipFakültesi; (Thesis) 1998.*

11. *Çakir Z, Şahin A. Acil servise başvuran adli nitelikteki sarsinti vakalarinin geriye dönük analizi, Atatürk Üniversitesi Tip Fakültesi Acil Tip Anabilim Dali, Erzurum*

12. *Akoğlu H, Denizbaşi A, Ünlüer E, Güneysel Ö, Onur Ö. Demografic Charactersitics of Trauma Patients of The Emergency Department of Marmara University Hospital. Marmara Medical Journal 2005; 18(3): 113-122.*

13. *Sunay YM, Serpil A. Zehirlenme olgularinin profili. Adli Toksikoloji Dergisi 2003: 1(1): 47-52.*

14. *Kiyan S, Özsaraç M, Ersel M, Yürüktümen A, Karahalli E, Özçete E et al. Analysis of patients with acute intoxication in a two year period. Turk J Emerg Med 2009; 9(1): 24-30.*

15. *Altun G, Azmak D, Yilmaz A, Yilmaz G. Trakya Üniversitesi Tip Fakültesi Acil Servisi'ne başvuran adli olgularin özellikleri. Adli Tip Bülteni 1997; 2(2): 62-6.*

16. *Çinar O, Acar YA, Çevik E, Kiliç E, Bilgiç S, Ak M. Analysis of Forensic Cases In The 0-18 Years Age Group That Were Presented to Emergency Department. Anatol J Clin Investig 2010; 4(3): 148-151.*

17. *Girgin V, Kocabiyik A. Is described the "predispozed to traffic accidents", who are the risky drivers? Düşünen Adam: The Journal of Psychiatry and Neurological Sciences 2003; 16(1): 35-38.*

18. *Bener A, Absood GH, Achan NV, Sankaran-Kutty M. Road traffic injuries in Al-Ain City.J R Soc Health 1992 Dec; 112(6): 273-6.*

19. *Al Suwaidi J, Bener A, Gehani AA, Behair S, Al Mohanadi D, Salam A et al. Does the circadian pattern for acute cardiac events presentation vary with fasting? J Postgrad Med 2006; 52(1): 30-3; discussion 33-4*

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Repairing large skin defects after thyroidectomy: Two cases report and review of literature

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Abstracts

Differentiated thyroid cancer typically has relatively good prognosis, but may invade surrounding tissues/organs, including great vessels, trachea, esophagus, recurrent laryngeal nerve and skin in the neck. Repairing large skin defects after thyroidectomy is a challenge. Five types of flap have been used for this purpose: a pedicled latissimus dorsi flap, a thin abdominal free flap, a one-edge thick flap in anterior thoracic region, a two-edge thick flap in anterior thoracic region and a deltopectoral flap. Here we report two cases of differentiated thyroid cancer with large skin defects after thyroidectomy treated with a one-edge thick flap and a two-edge thick flap in anterior thoracic region, respectively. These types of flap are good alternative to deltopectoral flap in repairing large skin defects in the neck.

Key words: large skin defects, thyroidectomy

Introduction

Thyroid cancer is the most common endocrine malignancy and accounts for about 1% of all cancers in USA[1]. It has four pathological types, undifferentiated carcinoma, poorly differentiated medullary carcinoma, well differentiated follicular carcinoma and papillary carcinoma. Papillary carcinoma is the most common pathological type accounting for about 80-85% of all thyroid cancers. It glows slowly and its prognosis is excellent. The 25-year survival rate is more than 95%[2]. Even without treatment, distant metastasis to the lungs and bone is rare[3], but regional lymph nodes metastasis and local invasion are common. Local tissues around thyroid contain the great vessels, trachea, esophagus and recurrent laryngeal nerve in the neck. However, skin invasion is rare and large skin invasion is more rare in clinical practice[4]. A systemic search of the literature from 1998 identified only 8 papers reporting 14 cases pf thyroid cancers with skin invasion, mostly in developing countries (Table 1)[4-11]. Large skin defects could be treated with a pedicled latissimus dorsi flap, a thin abdominal free flap, a one-edge thick flap in anterior thoracic region, a two-edge thick flap in anterior thoracic region and a deltopectoral flap. Here we reported a similar surgical treatment for two cases of thyroid cancer with large area skin invasion.

Case report 1

A 61-year-old woman with no family history of thyroid or other endocrine diseases presented with a necrotizing, ulcerated mass in anterior neck. This mass was noted by the patient 3 years ago, and was smaller and not ulcerated at the time. She did not notice it until ulceration occurred 18 months ago. Interestingly, the mass would shrink

Table 1. 14 thyroid cancers with skin invasion or metastasis were reported from 1998

Published year	Numbers of case	Published country	References
1998	1	USA	[4]
2004	4	Canada	[11]
2005	2	Malaysia	[5]
2007	3	India	[6]
2008	1	India	[7]
2008	1	India	[8]
2009	1	Korea	[9]
2010	1	USA	[10]

and skin ulcer would heal after discharging liquid from the ulceration. However, she still ignored it until ulceration become more serious and the mass grew rapidly several days before seeking medical advice (Figure 1A). This patient reported a history of partial thyroidectomy for papillary thyroid carcinoma (PTC) 5 years ago. However, no details of this surgery were available. At this time the serum thyroglobulin (TG) was 223.5ng/ml. Ultrasonography and computed tomography scan revealed a 13 by 10 cm mass in the anterior cervical region and several enlarged lymph nodes(Figure 1C). But chest x-ray and computed tomography scan confirmed that there was no distant metastasis. According to the results of preoperative examinations, surgical removal of all lesions was difficult since the tumor infiltrated the adjacent vital structures. Thyroidectomy and lymphadenectomy of the neck were performed with removal of most of the lesions, which revealed papillary thyroid carcinoma. We chose a two-edge thick flap in anterior thoracic region to repair it (Figure 1B). After the operation, the patient was euthyroid and under suppression therapy with levothyroxine. Three months later the patient was still alive and local control of the disease was achieved.

Case report 2

A 76-year-old woman with no family history of thyroid or other endocrine diseases presented with a necrotizing, ulcerated, anterior neck mass. This mass was noted by the patient 3 years ago, and was smaller and no ulceration at this time. She went to seek for medical advice when the mass grew rapidly half year ago, but refused the operation advice. She went to seek for medical advice three days ago when the mass invaded the skin of neck, ulcerated and bleeding (Figure 2A). This patient reported a history of partial thyroidectomy for papillary thyroid carcinoma (PTC) 11 years ago. However, no details of this surgery were available. At this time the serum thyroglobulin (TG) was 38.4ng/ml. Computed tomography scan revealed a 8 by 7 cm mass of left thyroid in the anterior cervical region and several enlarged lymph nodes (Figure 2C). But chest x-ray and computed tomography scan confirmed that there was no distant metastasis. Video laryngoscope examination revealed left vocal cord paralysis. Fine needle aspiration revealed dysplasia cells. According to the results of preoperative examinations, surgical removal of all lesions was difficult since the tumor

Figure 1. One huge recurrent thyroid cancer with skin invasion. A: a general picture preoperation. B: a general picture postoperation. C: a CT picture preoperation

Figure 2. Another huge recurrent thyroid cancer with skin invasion. A: a general picture preoperation. B: a general picture postoperation. C: a CT picture preoperation

infiltrated the adjacent vital structures. Palliative resection of the left thyroid carcinoma was performed with removal of most of the lesions, which revealed papillary thyroid carcinoma. We chose a one-edge thick flap in anterior thoracic region to repair it (Figure 2B).

Discussions

A huge thyroid cancer is usual to invade the surrounding tissue, but skin invasion is rare (Figure 1 and 2). If there is invasion of the skin, the skin must be removed. Therefore, it is an important problem to repair the skin defects. After resection of the cancer, there was large area of skin defects with trachea, esophagus, jugular vein, carotid artery and vagus nerve exposed. It is necessary to repair the skin defects, otherwise it is easy to get infected and threaten trachea, esophagus, jugular vein, carotid artery and vagus nerve. Which flap is the best choice to repair the skin defects? We will introduce advantage and disadvantage of five types of flap in the following (Table 2).

1 a pedicled latissimus dorsi flap (area B in the figure 3 A1)

Latissimus dorsi muscle is flat and wide. The blood supply of its inside part is the lower branch of the thoracodorsal artery. It is usually used for breast reconstruction on breast conservation surgery for breast cancer patients [12, 13]. But for the both patients, the skin defects were in anterior region of neck. It was far away from thoracodorsal artery. If the pedicled latissimus dorsi flap was used to repair the skin defects, the thoracodorsal artery might distort and obstruct, and lead to the latissimus dorsi flap ischemia and necrosis. Therefore, the pedicled latissimus dorsi flap was not appropriate to repair the skin defects in anterior region of neck.

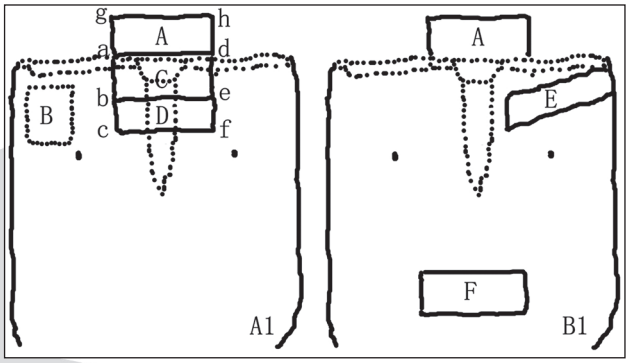

Figure 3. A schematic diagram of five different types of flap used to repair skin defects in the neck after thyroidectomy.
A: skin defects in the neck after thyroidectomy. B: a pedicled latissimus dorsi flap. C: a one-edge thick flap in anterior thoracic region. C and D: a two-edge thick flap in anterior thoracic region. E: a deltopectoral flap. F: a thin abdominal free flap.

2 a thin abdominal free flap (area F in the figure3 B1)

Abdominal skin is slack, especially in elderly women. A thin abdominal free flap is usually used to repair small area of skin defects, such as the breast skin defects after mastectomy[14]. But for the both patients, there was a larger area of skin defects in anterior region of neck. The transplanted thin abdominal free flap was not easy to survive only by local tissue fluid nutrition. Therefore, the thin abdominal free flap was not suitable.

Table 2. The difference among five types of flap to repair a large area of skin defects in the neck

Flap name	Blood supply	disadvantage	adoption
A pedicled latissimus dorsi flap	thoracodorsal artery	the thoracodorsal artery may distort and obstruct, and make it ischemia and necrosis	no
A thin abdominal free flap	no vessels	The flap has not enough blood supply.	no
A one-edge thick flap in anterior thoracic region	unilateral branches of thoracoacromial artery	Its blood supply is relatively inadequate to support a large flap.	Yes
A two-edge thick flap in anterior thoracic region	bilateral branches of thoracoacromial artery	Its activity is relative small.	yes
A deltopectoral flap	perforating branches of internal mammary artery	Its remote edge may necrosis and bad cosmetic result	no

3 a one-edge thick flap in anterior thoracic region (area C in the figure3 A1)

The anterior thoracic region is near the anterior region of neck. It is easy to get a flap to repair skin defects in the anterior region of neck. Activity of one-edge thick flap is large compared to a two-edge thick flap in anterior thoracic region. Its blood supply depends mainly on subcutaneous small blood vessels from unilateral branches of thoracoacromial artery. A large one-edge flap may lead to remote ischemia, and therefore this type of flap was used to repair the skin defects of the second patient but not suitable for the first patient.

4 a two-edge thick flap in anterior thoracic region (area C+D in the figure3 A1)

Compared with a one-edge flap, blood supply of a two-edge flap is better and can guarantee the survival, but its activity was smaller. Therefore, it was necessary to elevate part of the skin under the chin (area above line gh in the figure3 A1), transversely cut the lower edge (line be in the figure3 A1) of the two-edge thick flap and elevate the skin under the lower edge (area D in the figure3 A1). Then, sutured the skin under the chin (line gh) with the upper edge (line ad) of the two-edge thick flap together, and sutured the lower edge (line be) of the two-edge flap with the skin (area D) under the lower edge. Blood supply of the two-edge thick flap is from bilateral branches of thoracoacromial artery and its area is enough. Therefore, we used the flap to repair the skin defects of the first patient.

5 a deltopectoral flap (area E in the figure3 B1)

A deltopectoral flap was first described by Bakamjian in 1965[15]. It is above the pectoralis major muscle. Its internal edge is parasternal line and external edge is deltoid leading edge. Its internal blood supply is from perforating branches of internal mammary artery, and its external blood supply is from branches of thoracoacromial artery. Its flexibility is large and it can extend to any site in the neck even up to the level of zygoma. Therefore, it is usually used to repair a large area of skin defects in the neck or head[16]. However, it is a middle outward oblique flap. Its pedicle is easy to reverse when it is used to repair the skin defects in the above two cases, which may affect the blood supply of its remote edge and cause bad cosmetic result[17, 18]. Therefore, we rejected the deltopectoral flap.

After several days of observation, the one-edge thick flap for the second patient and the two-edge thick flap for the first patient survived with no infection and necrosis. The one-edge thick flap is a part of the two-edge thick flap. The one-edge thick flap area is half of the two-edge thick flap. Both of them have similar blood supply and are used to repair skin defects after thyroidectomy in the neck. The first patient had bilateral thyroid cancer and bilateral skin defects in the neck. The second patient only had left thyroid cancer and unilateral skin defects. Therefore, the one-edge thick flap was used to repair skin defects for the second patient and the two-edge thick flap for the first patient.

Conclusions

Thyroid cancer with large area of skin invasion or metastasis is rare. It is still a challenge for surgeon to repair skin defects after thyroidectomy. We compared the disadvantage and advantage among five different types of flap, a pedicled latissimus dorsi flap, a thin abdominal free flap, a one-edge thick flap in anterior thoracic region, a two-edge thick flap in anterior thoracic region and a deltopectoral flap. The deltopectoral flap is a good choice to repair skin defects in the neck and head from 1965. We introduced a one-edge thick flap and a two-edge thick flap in anterior thoracic region and repaired two cases of large skin defects after thyroidectomy with them. However, their late complications need to be investigated.

Acknowledgments

The patients were informed and agreed to publish the pictures in any scientific journals.

References

1. *Siegel R, Naishadham D, Jemal A: Cancer statistics, 2012. CA Cancer J Clin 2012; 62(1): 10-29.*

2. *LiVolsi VA: Papillary thyroid carcinoma: an update. Mod Pathol 2011; 24 Suppl 2: S1-9.*

3. *Song HJ, Xue YL, Xu YH, Qiu ZL, Luo QY: Rare metastases of differentiated thyroid carcinoma: pictorial review. Endocr Relat Cancer 2011; 18(5): R165-174.*

4. *Koller EA, Tourtelot JB, Pak HS, Cobb MW, Moad JC, Flynn EA: Papillary and follicular thyroid carcinoma metastatic to the skin: a case report and review of the literature. Thyroid 1998; 8(11): 1045-1050.*

5. *Harjit KD, Hisham AN: Large fungating thyroid cancers: a unique surgical challenge. Asian J Surg 2005; 28(1): 48-51.*

6. *Varma D, Jain S, Khurana N: Papillary carcinoma of thyroid presenting with skin ulceration. Cytopathology 2007; 18(4): 269-271.*

7. *Kim HS, Cha SH, Kim HO, Park YM: Cutaneous metastasis of thyroid cancer presenting as a nodulocystic mass with ulceration. J Dermatol 2009; 36(10): 559-560.*

8. *Harish K: Thyroid carcinoma with discharging sinus - a rarity: a case report. J Med Case Rep 2008; 2: 64.*

9. *Harish K, Murali S, Kilara N, Mahesh M, Manjunath CS: Cutaneous ulceration in thyroid lymphoma. Thyroid 2008; 18(8): 909-910.*

10. *Khan OA, Roses DF, Peck V: Papillary thyroid carcinoma metastatic to skin may herald aggressive disease. Endocr Pract 2010; 16(3): 446-448.*

11. *Alwaheeb S, Ghazarian D, Boerner SL, Asa SL: Cutaneous manifestations of thyroid cancer: a report of four cases and review of the literature. J Clin Pathol 2004; 57(4): 435-438.*

12. *Bonomi S, Salval A, Sorbi F, Settembrini F: To sever or not the thoracodorsal nerve in latissimus dorsi flap breast reconstruction. Plast Reconstr Surg 2012; 130(2): 367e-368e.*

13. *Lee KT, Lim SY, Pyun JK, Mun GH, Oh KS, Bang SI: Improvement of upper extremity lymphedema after delayed breast reconstruction with an extended latissimus dorsi myocutaneous flap. Arch Plast Surg 2012; 39(2): 154-157.*

14. *Yang SJ, Eom JS, Lee TJ, Ahn SH, Son BH: Recipient vessel selection in immediate breast reconstruction with free abdominal tissue transfer after nipple-sparing mastectomy. Arch Plast Surg 2012; 39(3): 216-221.*

15. *Bakamjian VY: A two-stage method for pharyngoesophageal reconstruction with a primary pectoral skin flap. Plast Reconstr Surg 1965; 36: 173-184.*

16. *Krijgh DD, Mureau MA: Reconstructive Options in Patients With Late Complications After Surgery and Radiotherapy for Head and Neck Cancer: Remember the Deltopectoral Flap. Ann Plast Surg 2012.*

17. *Krizek TJ, Robson MC: Potential pitfalls in the use of the deltopectoral flap. Plast Reconstr Surg 1972; 50(4): 326-331.*

18. *Gingrass RP, Culf NK, Garrett WS, Mladick RA: Complications with the deltopectoral flap. Plast Reconstr Surg 1972; 49(5): 501-507.*

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Clinical features of perianal hidradenoma papilliferum: Three cases and a literature review

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Abstract

Due to a lack of distinctive clinical features, the diagnosis of hidradenoma papilliferum is still primarily based on the postoperative histopathologic examination. Although it is actually a rare benign tumor, surgery is required because it can become malignant. However, hidradenoma papilliferum in the perianal area can occur simultaneously with other perianal diseases such as asymptomatic external hemorrhoids and skin tags, for which expectant treatment is recommended. Thus, treatment for perianal hidradenoma papilliferum will be delayed if it cannot be diagnosed in time. Therefore, it is important to recognize the clinical features of perianal hidradenoma papilliferum. In this study, we present 3 cases of perianal hidradenoma papilliferum and review the literature to emphasize its clinical characteristics, which would assist proctologists in making the correct diagnosis.

Key words: Perianal hidradenoma papilliferum, clinical features.

Introduction

Hidradenoma papilliferum is a rare benign tumor that has been reported to arise in a number of special sites, including the scalp [1], nasal skin [2], vulva [3], and trunk [4]. Most studies have focused on its pathological characteristics. The lesion is characterized by multiple tubular or cystic cavities covered with 2 or 3 layers of columnar epithelial cells and evidence of apocrine secretion [5]. However, due to a lack of complete understanding of its clinical features, it is still difficult to make correct clinical diagnosis prior to histopathologic examination. In other words, hidradenoma papilliferum is usually diagnosed after surgery. Although it is in fact a benign tumor, surgery is recommended because it could develop into a malignant tumor, according to the literature[6]. However, perianal hidradenoma papilliferum can occur simultaneously with other perianal diseases. Expectant treatment is recommended for some of these diseases, such as asymptomatic external hemorrhoids and skin tags[7]; thus, treatment for perianal hidradenoma papilliferum will be delayed if it is not be diagnosed in time. Therefore, clinical awareness of perianal hidradenoma papilliferum is important. We present 3 cases of perianal hidradenoma papilliferum and review the literature to elucidate its clinical characteristics, which would assist proctologists in making the correct diagnosis.

Case Reports

Case 1 was a 46-year-old woman who presented with a 2-month history of a painless mass with seepage. Proctologic examination revealed that a 0.5 × 0.5 cm firm yellow-white nodule with an erosive surface was located 1 cm from the right anal margin. It appeared that there was a tenuous subcutaneous track between the nodule and the anus. There was no history of itching, bleeding, fever, or purulent discharge. Anoscopy was normal. She was diagnosed with an anal fistula. During the operation, the nodule was excised with a narrow margin. However, no internal opening was found after incision of the suspicious fistula. The wound was left open, and it had healed at 12 weeks after the operation. The postoperative diagnosis by the pathologist was perianal hidradenoma papilliferum, which was composed of multiple tubular or cystic cavities covered with 2 layers of columnar cells and evidence of apocrine secretion (Figure 1a). No recurrence was observed for 56 months.

Case 2 was a 45-year-old woman complaining of a 10-year history of a protruding mass around the anal margin that was occasionally accompanied by fresh blood in the stool. Physical examination revealed a skin tag located in the left posterior

quadrant of the anal margin. There was a 0.7 × 0.5 cm firm, freely mobile reddish nodule with a smooth surface on the top of this tag. Anoscopy revealed hemorrhoidal mucosal hyperemia on the corresponding site. Digital examination was normal. She was diagnosed with a mixed hemorrhoid clinically, and she underwent hemorrhoidectomy. The pathological report was indicative of perianal hidradenoma papilliferum (Figure 1b). Her wound had healed at 6 weeks after the operation. No recurrence was observed for 39 months.

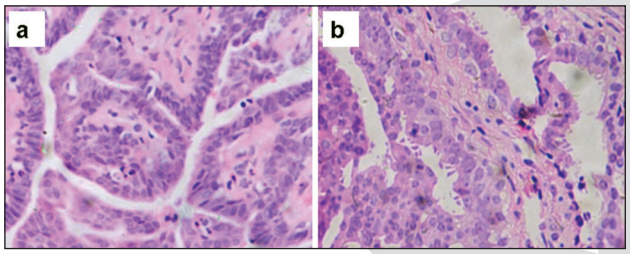

Figure 1. Higher magnification revealing columnar eosinophilic cells with apocrine differentiation (hematoxylin and eosin stain, ×200). a) Case 1; b) Case 2

Case 3 was a 30-year-old woman who complained of a more than 1-year history of a painless mass with itching (Figure 2a). There was no history of bleeding, fever, or purulent discharge. Physical examination revealed a 0.4 × 0.5 cm firm, freely mobile yellow-white nodule with a smooth surface located in the right anal margin. Digital examination and anoscopy were normal. She was diagnosed with a perianal nodule that was considered perianal hidradenoma papilliferum. Local excision was performed, and the wound was sutured. The wound had healed 2 weeks after the resection (Figure 2b, 2c). Lastly, the clinical diagnosis was confirmed by the pathological examination (Figure 2d). No recurrence was observed for 5 months.

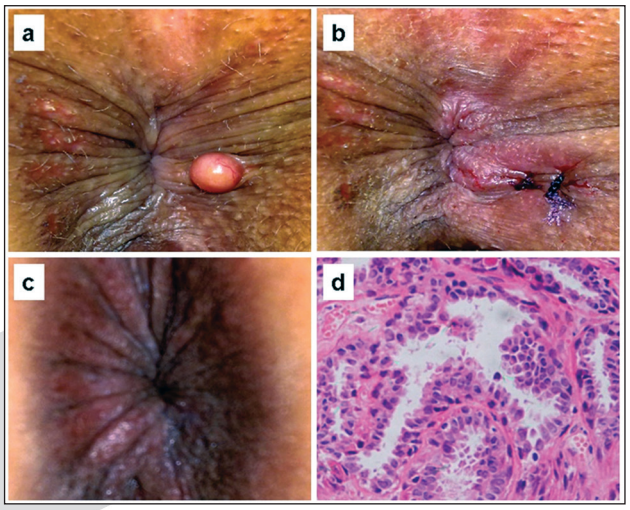

Figure 2. Preoperative and postoperative perianal appearance as well as pathological data of Case 3. a) A 0.4 × 0.5 cm yellow-white nodule in the right perianal area before surgery; b) the sutured wound; c) the healed surgical wound at 2 weeks after resection; d) pathological data from higher magnification (hematoxylin and eosin stain, ×200)

Discussion

Hidradenoma papilliferum of the perianal region was previously believed to arise from the apocrine gland; however, it has recently been accepted as originating from mammary-like glands [8]. Due to a lack of complete understanding of its clinical manifestation, it is difficult to make a clinical diagnosis prior to the pathological examination. However, the current report might change this situation.

Based on our cases and those in the literature, perianal hidradenoma papilliferum usually occurs in women 30–60 years of age and occasionally in younger or older patients [9]. At this point, reports of this lesion in male patients are very rare [10]. The lesion is typically a small, protruding nodule with a colorful appearance. It is firm and freely mobile with a clear margin and smooth surface that occasionally may be ulcerated, as described in Case 1. It is usually asymptomatic, but occasionally is accompanied by itching, pain, or discharge if it ulcerates [6,9]. Among these features, the most important are the colorful appearance and lack of symptoms. Based on these features, we correctly diagnosed Case 3 prior to pathological examination.

In addition, as mentioned in Case 2, perianal hidradenoma papilliferum and other anal diseases such as hemorrhoids and anal fistulas can occur simultaneously, which can further confound diagnosis and treatment. When perianal hidradenoma papilliferum occurs with other asymptomatic anal disorders, a missed diagnosis should be avoided. Certainly, misdiagnosis should also be avoided for patients with perianal hidradenoma papilliferum alone, as observed for Case 1. It must be differentiated from other conditions in the perianal area, including thrombosed external hemorrhoids, anal abscess, viral warts, endometriosis, angioma, and melanoma [5,9]. Both missed diagnoses and misdiagnoses can affect the clinical decision, which could result in patient harm.

In conclusion, perianal hidradenoma papilliferum is an uncommon benign disease that should be carefully differentiated from other perianal disorders. If the terminology as well as clinical features of perianal hidradenoma papilliferum are kept in mind, it should be not difficult to make a correct diagnosis clinically prior to pathologic examination. If diagnosed, it must be excised with a narrow margin as curative therapy.

Acknowledgments

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References

1. *Moon JW, Na CH, Kim HR, Shin BS. Giant ectopic hidradenoma papilliferum on the scalp. J Dermatol. 2009; 36: 545-7.*
2. *Lee HJ, Lee D, Jung SY, Hong SK, Seo JK, Sung HS. Hidradenoma papilliferum occurring on the nasal skin. Ann Dermatol. 2011; 23: S254-7.*
3. *Duhan N, Kalra R, Singh S, Rajotia N. Hidradenoma papilliferum of the vulva: case report and review of literature. Arch Gynecol Obstet. 2011; 284: 1015-7.*
4. *Morimura S, Kadono T, Sugaya M, Sato S. Ectopic hidradenoma papilliferum on the abdomen. Eur J Dermatol. 2011; 21: 278-9.*
5. *Elder D, Elenitsas R, Ragsdale BD. Hidradenoma palilliferum. In: Elder D, Elenitasis R, Jaworsky C, Johnson B Jr, eds. Lever's histopathology of the skin. 8th ed. Philadephia: Lippincott-Raven; 1997: 770-1.*
6. *MacNeill KN, Riddell RH, Ghazarian D. Perianal apocrine adenocarcinoma arising in a benign apocrine adenoma; first case report and review of the literature. J Clin Pathol. 2005; 58: 217-9.*
7. *Rivadeneira DE, Steele SR, Ternent C, Chalasani S, Buie WD, Rafferty JL. Practice Parameters for the Management of Hemorrhoids (Revised 2010). Dis Colon Rectum. 2011; 54: 1059 –1064.*
8. *Parks A, Branch KD, Metcalf J, Underwood P, Young J. Hidradenoma papilliferum with mixed histopathologic features of syringocystadenoma papilliferum and anogenital mammary-like glands: report of a case and review of the literature. Am J Dermatopathol. 2012; 34: 104-9.*
9. *Handa Y, Yamanaka N, Inagaki H, Tomita Y. Large ulcerated perianal hidradenoma papilliferum in a young female. Dermatol Surg. 2003; 29: 790-2.*
10. *Loane J, Kealy WF, Mulcahy G. Perianal hidradenoma papilliferum occurring in a male: a case report. Ir J Med Sci. 1998; 167: 26-7*

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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.

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Introduction

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Instructions for the authors

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Table 1. Page layout description

Paper size	A4
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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)

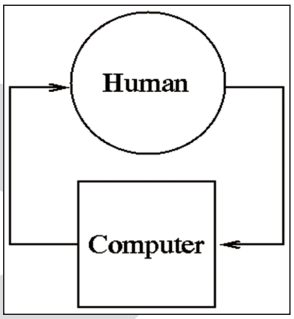

Figure 1. Text here

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.*
2. *Stewart SM, Lam TH, Beston CL, et al. A Prospective Analysis of Stress and Academic Performance in the first two years of Medical School. Med Educ 1999; 33(4): 243- 50.*

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