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Seasonal variation of whole blood viscosity and hematocrit in China

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Abstract

Background: Hemorheological variables play important roles in influencing the risk for cardio-cerebrovascular diseases. The morbidity and mortality of cardio-cerebrovascular diseases in Northeast China are much higher in winter than in summer. This study aimed to investigate the seasonal variations of relevant hemorheological parameters in healthy subjects.

Methods: Ninety-two healthy subjects of various ages were recruited in the present study. Hemorheological variables, such as whole blood viscosity, plasma viscosity, hematocrit and plasma fibrinogen level, were measured.

Results: Whole blood viscosity and hematocrit were significantly higher in the winter compared with those in summer. However, there were no significant seasonal differences in plasma viscosity and fibrinogen. The winter low-shear blood viscosity was consistently higher than that in the summer irrespective of age in an adult healthy population.

Conclusion: Thus, seasonal factors may markedly affect whole blood viscosity and hematocrit levels in healthy subjects in Northwest China.

Key words: Hemorheology, healthy population, seasonal variation.

Introduction

Cardiovascular and cerebrovascular diseases are the most common causes of mortality and morbidity in China. Interestingly, the mortality and morbidity associated with these diseases are markedly higher in cold months (October to January) than the other months. (1) Elderly patients are more sensitive to the climatic or seasonal variation.(2) Of note, cardio-cerebrovascular diseases are commonly concomitant with the increased plasma viscosity, and hemorheological variations are typically present even before the patient is symptomatic. Hemorheological parameters, especially plasma fibrinogen, are strong risk factors for myocardial infarction and stroke.(3) Moreover, these parameters may be sensitive to factors, such as diet, physical exercise and mental stress. In Northeast China, there are dramatic seasonal variations in air temperature ranging from 33°C to -30°C. Thus, the cold weather may contribute to the marked increase in relevant hemorheological parameters, which may play a contributing role in the risk for cardio-cerebrovascular diseases.

Cardio-cerebrovascular diseases present, not only in elderly patients, but also in young adult subjects. However, previous studies have primarily focused on the seasonal variations of hemorheological indexes in elderly subjects, whereas studies reporting on youth and middle-aged healthy subjects are relatively rare.(4) We therefore observed the seasonal variations of hemorheological parameters in adult (middle-aged) healthy subjects of different ages to elucidate the relationship between these variables and the potential risk for cardio-cerebrovascular diseases in Northeast China. Addressing this issue would help support the importance of health education in populations of cold areas in China.

Materials and methods

Subjects

Ninety-two healthy volunteers (46 women and 46 men) were recruited in the present study. The enrolled subjects were determined to be healthy on the basis of their medical history and physical and laboratory examinations. The subjects were non-obese, non-smokers, and had normal blood pressure, blood glucose and lipid profiles. The subjects were grouped into a youth group (aged 20 to 44, 30 subjects), a middle-aged group (aged 45 to 59, 30 subjects), and two elderly groups (aged 60 to 70, 20 subjects; aged more than 70, 12 su-

bjects). All enrolled subjects provided written informed consent. The present study was approved by the ethic commission of the First Clinical college, Harbin Medical University in China.

Measurement of hemorheological variables

Hemorheological measurements were performed in June to July of 2011 (summer) and November to December of 2011 (winter). Blood samples were obtained from an antecubital vein between 8 am and 9 am after nocturnal fasting. The samples were transported to the laboratory for analysis within two hours of collection. Blood hemorheology (>100mpa·s was defined as high-shear viscosity; <50mpa·s was defined as low-shear viscosity), hematocrit, plasma viscosity, and plasma fibrinogen concentration were determined as previously described.(5)

Statistical analyses

Results are presented as mean \pm standard deviation (SD). Data comparisons made between and within groups were analyzed using two-tailed Student's t- test. A p < 0.05 was considered statistically significant. All statistics were calculated using SPSS 11.5 software (SPSS, Munich, Germany).

Results

Baseline characteristics and hematological variables

No significant seasonal differences were observed for baseline body mass index, blood pressure, blood glucose, total cholesterol and triglyceride levels in the enrolled healthy subjects (all p >0.05, Table 1).

| Variables | In Summer (n-92) | In Winter (n-92) | |
|-------------------------------------|---------------------|---------------------|--|
| Age (years) | 48± | 26 | |
| Female (%) | 46 (50%) | | |
| Body mass index(kg/m ²) | 22.6±0.55 | 22.4±0.58 | |
| Blood pressure (mmHg) | | | |
| systolic blood pressure | 90±15 | 91±14 | |
| diastolic blood pressure | 66±13 | 65±14 | |
| Plasma glucose(mmol/L) | 4.90±0.62 | 4.81±0.59 | |
| Total cholesterol (mmol/L) | 4.53±1.02 | 4.55±1.07 | |
| Triglycerides (mmol/L) | 1.36±0.82 | 1.38±0.86 | |

Table 1. Baseline demographic and hematological variables

Low-shear viscosity in the two elderly groups (60-69 years: 9.72±1.73 mPas; >70 years: 9.16±1.57 mPas) were lower compared with the youth group $(10.56\pm1.44 \text{ mPas}, \text{ both } p<0.05)$ and the middleaged group (10.44±1.70 mPas, both p<0.05). Conversely, the high-shear viscosity was slightly higher in the middle-aged and elderly subjects (45-59 years: 5.08±0.48 mPas, 60-69 years: 4.77±0.63 mPa·s, >70 years: 4.94±0.40 mPa·s) compared with the young adult group $(4.49\pm0.63 \text{ mPa}\cdot\text{s})$. Additionally, the elderly population had a greater high-shear viscosity compared with the young adult population (aged 60 to 70: p=0.03; aged more than 70: p=0.001). Furthermore, the plasma viscosity in the youth group was significantly lower compared with the middle-aged and elderly groups (p < 0.001). However, there was no significant difference in hematocrit and plasma fibrinogen among the recruited subjects, regardless of subject age.

Hemorheological variables in the winter and summer

In all patients, hematocrit (Figure 4), high-shear viscosity (Figure 3) and low-shear viscosity (Figure 2) were significantly affected by seasonal variation, with the three indices being higher in the winter season. In the winter, there was a non-significant trend towards a higher plasma fibrinogen level compared to levels observed in the summer (Figure 1). There were no differences in plasma viscosity (Figure 5) between the winter and summer.



Figure 1. Seasonal changes of plasma fibrinogen in healthy populations of various ages.

| A | Patients Blood viscosity (mPas) | | Plasma viscosity | Hematocrit | FIB | |
|-------|---------------------------------|---------------|------------------|--------------|------------|-----------|
| Age | number | 1s-1 | 150s-1 | (mPas) | (%) | (g/L) |
| 20-44 | 30 | 10.56±1.44 | 4.49±0.63 | 1.46±0.12 | 44.16±2.38 | 3.01±0.68 |
| 45-59 | 30 | 10.44±1.70 | 5.08±0.48*** | 1.63±0.10*** | 44.67±3.07 | 3.08±0.69 |
| 60-69 | 20 | 9.72±1.73*# | 4.77±0.63*# | 1.65±0.10* | 44.55±3.40 | 3.18±0.72 |
| 70- | 12 | 9.16±1.57**## | 4.94±0.40** | 1.64±0.11** | 44.42±2.72 | 3.31±0.76 |

Table 2. Hemorheological variables in relation to age

* ** *** p < 0.05 (versus group aged 20 to 44), # ## p < 0.05 (versus group aged 45-59)



Figure 2. Seasonal changes of low-shear viscosity in healthy populations of various ages.



Figure 3. Seasonal changes of high-shear viscosity in healthy populations of various ages.



Figure 4. Seasonal changes of hematocrit in healthy populations of various ages.



Figure 5. Seasonal changes of plasma viscosity in healthy populations of various ages.

Discussion

Previous studies have demonstrated positive correlations between air temperature and high-shear viscosity, as well as air temperature and plasma viscosity.(5) Conversely, there was an inverse relationship between air temperature and low-shear viscosity in patients with cerebral infarction.(6) The present study enrolled healthy subjects to investigate seasonal changes in hemorheological parameters, as well as determine the effect of age. The adrenergic system is involved in the regulation of platelet aggregation and blood viscosity. Studies have shown that the adrenergic system may play a role in the higher incidence of sudden cardiac death during cold months.(7) The activation of the sympathetic nervous system resulting from colder weather has been shown to be an important risk factor for sudden cardiac death, primarily through promoting circadian rhythm in patients without coronary artery disease(8). However, serum adrenaline and norepinephrine levels were not measured in the current study. Thus, we still did not confirm the impact of the change in air temperature on the sympathetic nervous system and blood viscosity.

The present study revealed that in cold months there was a pronounced improvement in whole blood low-shear viscosity, which was especially evident in the >70 years old group. In other words, a decrease in air temperature was correlated with an increase in whole blood low-shear viscosity. This finding may be a consequence of tissue hypoxia, resulting from a decrease in air temperature, which can stimulate sympathetic nerve activity and promote erythrocytosis. Increased numbers of red blood cells can promote platelet adhesion, most likely due to the increased number of platelet impacts with the vessel wall. Increased whole blood viscosity may facilitate complete vessel occlusion following the formation of a platelet thrombus on the vessel wall.(8-10)

Cold-induced activity of sympathetic nerves can induce the release of sequestered platelets and/or the production of new platelets, which are generally larger than older platelets. Large platelets tend to aggregate and adhere more readily to blood vessels compared with small platelets.(11, 12) Erythrocyte deformability has been demonstrated to be influenced by platelet activation, which has been shown to lower blood viscosity.(13) In the present study, we found that high-shear blood viscosity in the middle-aged subjects was slightly higher than the elderly population, which may be associated with greater outside physical training and a greater probability of exposure to cold air. Blood viscosity tends to decrease as the result of general physical activity.(14) A relationship between age and decreased endothelial function has been observed, which can reduce prostacyclin synthesis and compromise anti-thrombotic activity of the vascular wall due to impaired nitric oxide production, among other factors. These adverse effects can lead to diastolic dysfunction of the left ventricle and increased rigidity of vascular wall.(15) The high incidence of cold-related mortality in the elderly may be explained by the increased incidence of initial arterial disease, as well as their susceptibility to thrombosis due rheological changes, which may be harmless to young fit individuals. (16)

Hematocrit is regarded as an indicator of blood viscosity. Hematocrit in the winter was consistently higher than that in summer, regardless of gender. However, previous research has not shown an association between increased hematocrit and the incidence of adverse events.(7) Prior work has shown a significant seasonal pattern in hematocrit deferral rates that is associated with genetic background, environmental temperature, diet, age, and physical exercise.(17-19) High-sodium and high-fat diets, as well as low intake of fruit and vegetables, have been shown to accelerate the increase in hematocrit in cold areas of Northeast China. In addition, the decrease in physical training might further contribute to the increased hematocrit.(14)

Air pollution has been consistently associated with increased mortality and morbidity for cardiovascular disease. During the cold months in Northeast China, there are high concentrations of particulate matter and carbon monoxide due to air pollution. During periods of high air pollution, an increase in plasma viscosity has been observed both in men and women.(20) This increased blood viscosity can lead to an increase in shear stress, which in turn can act on the functional endothelium and promote the release of nitric oxide (NO) and other vasoactive mediators, causing vasodilation and lowering peripheral vascular resistance.(21) Seasonal blood viscosity variation could indicate impaired vascular regulation and the potential for increased cardiovascular and cerebrovascular risk.

Clinical trials and epidemiological observations have indicated that elevated plasma fibrinogen levels are strongly correlated with an increased frequency of vascular events. Moreover, seasonal variation in the concentration of plasma fibrinogen has been previously reported, with higher values occurring in the cold months of the year, most likely due to the colder air temperatures.(22) The increase of plasma fibrinogen concentration not only leads to an increased plasma viscosity, but also to a decreased zeta charge on the surface of red blood cells, which may induce aggregation of red cells.(23) Elevated plasma fibrinogen levels appear to be directly related to an increased risk of thrombotic events, indicating an association of seasonal thrombotic events with environmental temperature.(24, 25) In conclusion, blood viscosity and hematocrit in the winter was consistently higher than that in the summer in all age groups. Thus, seasonal factors may markedly affect whole blood viscosity and hematocrit.

Source of support

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Relationship between serum prohepcidin levels and serum transferrin receptors in children with thalassemia minor and iron deficiency anemia

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Abstract

Introduction and Aim: Hepcidin, synthesized from prohepcidin as a preprohormone, is a key peptide in regulating iron metabolism and iron distribution throughout the body. The relationship between serum prohepcidin levels and serum transferrin receptors in children with thalassemia minor and iron deficiency anemia were investigated in this study.

Materials and Methods: Two study groups, children diagnosed with iron deficiency anemia and beta thalassemia minor, and a control group of healthy children were included. The following information was collected for each patient: blood count, serum iron and iron binding capacity, ferritin levels, serum transferrin receptor levels, and C-reactive protein and prohepcidin levels.

Results: A total of 63 children were included in the study. No significant difference was found between the prohepcidin levels or the prohepcidin/ferritin rates of the three groups (p>0.05). The highest serum transferrin receptor level was in the anemia group, and the average serum transferrin receptor level was significantly higher for the anemia group than for the control group (p<0.001). However, no significant difference was observed between children with beta thalassemia minor and the other groups (p=0.15).

Conclusion: We determined that prohepcidin measurement does not reflect hepcidin level. Furthermore, prohepcidin is not a convenient clinical indicator. No significant differences in prohepcidin levels were observed between groups, and no correlation exists between prohepcidin level and serum transferrin receptor or other iron parameters. Based on our results, serum transferrin receptor level and serum transferrin receptor/ferritin rate appear to be more useful for identifying iron deficiency. Key words: Beta thalassemia minor, iron deficiency anemia, hepcidin, prohepcidin, serum transferrin receptor.

Introduction and aim

Thalassemia is a group of hemoglobin synthesis disorders characterized by the reduction or absence of synthesis of one or more globulin chains (1). Individuals who have only one of two beta thalassemia genes are considered as beta thalassemia carriers and diagnosed with beta thalassemia minor (BTM) (also known as, heterozygote beta thalassemia). Frequently, there are no clinical findings. Heterozygosity is generally recognized during hematological examination (2). Iron deficiency anemia (IDA) is an important public health matter for nursing children in developing countries, adolescents, pregnant women, and individuals in low socioeconomic circumstances. An estimated two billion people are affected by iron deficiency world-wide, and more than half have anemia (3). Differential diagnosis of both of BTM and iron deficiency anemia is important, because IDA causes microcytic anemia, frequently (4).

The number of transferrin receptors on the cell surface increases and decreases based on the cell's iron requirement (TfR). Serum transferrin receptors (sTfR) are transferrin receptors separated from the cell surface with 85 kDa and bound to circulating transferrin (5). Although the most frequent cause of increased serum transferrin receptor levels is a lack of iron erythropoiesis (6), sTfr levels are also elevated in thalassemia patients with increased erythropoietic activity, autoimmune hemolytic anemia and hereditary spherocytosis (7).

Hepcidin is a peptide that plays a key role in regulating iron metabolism and distribution in the body. Hepcidin also has antimicrobial properties as it is synthesized in the liver as a preprohormone. Prohepcidin is a leader peptide that is converted into hepcidin (8). While the production of liver hepcidin is suppressed by conditions such as hypoxia, decreased body iron storage, and, especially, increased erythropoietic activity, the production of hepcidin is increased by conditions such as inflammation and iron overload.

sTfR levels are elevated due to increased erythropoietic activity associated with both IDA and BTM, and hepcidin is suppressed by the increased erythropoietic activity. Previous studies have shown that hepcidin levels are reduced in patients with beta thalassemia major and IDA. To the best of our knowledge, no studies have evaluated hepcidin or prohepcidin levels in patients with BTM. The aim of our study was to determine the relationship between prohepcidin and sTfR levels.

Material and methods

Twenty children diagnosed with IDA, 21 children diagnosed with BTM, and 22 healthy controls were included in this study on children's health and diseases polyclinics. One parent of each child participating in the study was informed about the study and provided written informed consent.

A diagnosis of iron deficiency anemia was determined given a hemoglobin level ≤ 11 g/dl and a ferritin measurement of <10 ng/ml. Patients with hemoglobin A2 > 3.4% and ferritin ≥ 10 ng/ ml were diagnosed with β-thalassemia minor. Healthy children who are presented to the well-baby clinic for vaccinations or routine check-ups were included in the control group. Patients who had febrile illness within the last four weeks, recently used iron supplements, or who had C-reactive protein levels >8 mg/L were excluded from the study. All patients provided blood samples and blood smears, and the following information was obtained: complete blood count, serum iron concentration, total iron binding capacity, ferritin level, and CRP values. Patients' samples were evaluated on the same day. The blood samples drawn for sTfR and prohepcidin studies were centrifuged at 1500 rpm to separate the serum, and the serum samples were kept at -80°C until the date of the study.

Statistical Analysis

Data analysis was performed using the SPSS for Windows 11.5 package program. The Shapiro Wilk W test was used to determine whether continuous variables were normally distributed. Descriptive statistics were presented as average \pm standard deviation or median (interquartile width) for continuous variables, and categorical variables were presented as number of cases (percentage). Differences between the average values of the groups were evaluated using one-way ANOVA. Differences between the median values of the groups were evaluated using the Kruskal Wallis test. Post hoc Tukey or non-parametric multiple comparison tests were used to detect the specific group or groups causing statistically significant ANOVA or Kruskal-Wallis results. Categorical variables were examined using Pearson's chi-square test. Spearman's correlation test was used to evaluate correlations between continuous variables. To determine effectivity of rohepcidin and sTfR measurements, area under the ROC curve calculations were used to distinguish control and case groups. The best cutoff point was determined using the Youden index when the area under the curve was important. Also, the sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy levels for the cutoff point were calculated. A p-value less than 0.05 was considered statistically significant.

Results

Thirty female children (47.6%) and 33 male children (52.4%) participated in the study. The mean patient age was 7.1 ± 4.3 years (range: 2-15) years). The demographic characteristics of children who participated in the study are shown in Table 1. Hematological and biochemical results are shown in Table 2. Prohepcidin levels were similar among the three groups. No significant relationships between inter-group serum prohepcidin levels and other laboratory measurements were found. The iron deficiency anemia group had the highest sTfR levels, and the average sTfR level $(3.52 \pm 23\mu \text{g/ml})$ for the IDA group was significantly higher than that of the control group $(1.23 \pm$ 0.3µg/ml; p<0.001). No significant difference (2.4 \pm 0.9 mg/ml.) was observed between the BTM group and the control group (p=0.15). The average sTfR level for the thalassemia minor group was significantly higher than for the beta control group (p<0.001). The sTfR point of cut-off for the IDA group and the control group was at 1.75; the sensitivity for differential diagnosis, the positive predictive value (PPV), and the negative predictive value (NPV) were all 95%. The sTfR cut-off point was 1.54 for the thalassemia group and the control group; the corresponding sensitivity was 95%, specificity was 86%, the positive predictive value was 87%, and the negative predictive value was 95% (Table 3).

| Table | 1. | Patient | demograph | ic cl | haracteristics |
|-------|----|---------|-----------|-------|----------------|
|-------|----|---------|-----------|-------|----------------|

| | IDA Group (n=20) | BTM Group (n=21) | Control Group (n=22) |
|--------------------------|----------------------------------|---------------------|--------------------------------|
| Age (years) (minmax.) | 4.0±3.9 ^{a,b} (2-14) | 8.7±3.5 b (3-15) | 8.5±4.0 ^a (2-15) |
| Gender | | | |
| Female | 7 (35.0%) | 12 (57.1%) | 11 (50.0%) |
| Male | 13 (65.0%) | 9 (42.9%) | 11 (50.0%) |

^a The difference between the control group and the anemia group is statistically significant (p < 0.001).

^b The difference between the anemia group and the thalassemia group is statistically significant (p<0.001).

IDA: Iron deficiency anemia, BTM: Beta thalassemia minor

| Table 2. | Hematologic | and bioch | emical labora- |
|------------|-----------------|--------------|----------------|
| tory resul | ts in comparise | on to the co | ontrol group. |

| | Control | IDA | BTM |
|------------------------|----------------|-------------------------|-------------------------|
| | Group | Group | Group |
| RBC x104/µl | 4.8 ±0.32 | 4.6 ± 0.34 | 5.9 ±0.4 ^{a,x} |
| Hb (g/dl) | 13.4±0.9 | 9.7±1.2 ^a | 11.6±0.9 ^{a,x} |
| Htc (%) | 39.8±2.8 | 29.6±2.9 ^a | 37.4±5.3 ^x |
| MCV (fl) | 82.3±4.1 | 64±7.7 ^a | 61.7±3 ^a |
| MCH (pg) | 27.8±1.5 | 21.1±3.3 ^a | 19.5±0.9 a,z |
| MCHC (g/dl) | 33.8 ± 0.5 | 32.1±2.9 ^a | 31.7±0.6 a,y |
| RDW (%) | 12.7±0.7 | 18.9±2.9 ^a | 15.2±1.4 ^{a,y} |
| PLT (X 103) (µL) | 291.5±55.4 | 419.5±154 ° | 387±96.4 ª |
| WBC (X 103) (µL) | 7.0±1.9 | 8.4±1.8 ^b | 7.5±1.5 |
| Serum Iron (µg/dl) | 97.3±30.3 | 30.8±12.9ª | 75.1±30.5 °,x |
| TDBK(µg/dl) | 369.2±39.2 | 427.3±75.5 ^b | 354.3±42.9 × |
| TSI (%) | 26.7±9.5 | 7.4±3.6 ^a | 21.7±9.6 ^x |
| sTfR (µg/ml) | 1.23±0.3 | 3.52±2.3 ^a | 2.4±0.9 ^a |
| Ferritin (ng/ml) | 33.7±13.6 | 5.8±2.1ª | 38.5±21.1 x,y |
| Prohepcidin (ng/ml) | 73.5±26.1 | 75.9±32.4 | 77.2±25.6 |

- ^{*a*} When the control group was compared p < 0.001;
 - ^{*b*} when the control group was compared p < 0.005;
 - ^c when the control group was compared p=0.03;
 - *x* according to IDA p<0.001;
 - ^{*y*} as to IDA p<0.005;
 - ^z as to IDA p=0.02

Table 3. sTfR's diagnostic power for differential diagnosis

| Indicators | Control-IDA | Control-BTM |
|-------------|--------------------|--------------------|
| Number of | 42 | 43 |
| cases | 72 | 75 |
| Sensitivity | 19/20 (95.0%) | 20/21 (95.2%) |
| Specificity | 21/22 (95.5%) | 19/22 (86.4%) |
| Positive | | |
| Predictive | 19/20 (95.0%) | 20/23 (87.0%) |
| value | | |
| Negative | | |
| Predictive | 21/22 (95.5%) | 19/20 (95.0%) |
| Value | | |

When correlations between sTfR and other laboratory measurements were investigated, a significant negative correlations between sTfR and Hb (r=-0.86; p<0.001), Hct (r=-0.78; p<0.001), MCV (r=-0.87; p<0.001), MCH (r=-0.85; p<0.001), and MCHC (r=-0.73; p< 0.001), were detected in the IDA group. Significant positive correlations between sTfR and RDW (r=0.56; p=0.009), PLT (r=0.50; p=0.02), and TDBK (r=0.49; p=0.02) were also found in the IDA group. In the beta thalassemia minor group, sTfR was significantly correlated with Hct (r=-0.4; p=0.02), RDW (r=0.54; p=0.01), and PLT (r=0.45; p=0.03). The sTfR/ ferritin ratio was highest in the IDA group, followed by the BTM group and the control group, respectively (p<0.001). The Mentzer index was highest for the control group (17.2), and lowest for the thalassemia group (10.4); the index values were significantly different for all three groups (p<0.001).

Discussion

Differential diagnosis of IDA and BTM is important as they are frequent causes of microcytic anemia. In this study, we evaluated the efficacy of using sTfR levels, prohepcidin levels and the sTfR/ferritin ratio for differential diagnosis of BTM with IDA.

In a previous study, Lafferty et al. (10) reported that RBC count is the most reliable parameter for identifying BTM. In accordance with the literature, RBC values were significantly higher in the BTM group compared to the control and IDA groups in our study. RBC count is usually reduced in patients with iron deficiency. The IDA group's RBC values were lower than those of the control group in our study; the difference was not statistically significant.

MCV is an important parameter in thalassemia (11). A MCV value of 72 fl or lower has been shown to identify Thalassemia syndromes with maximum sensitivity and specificity (11). Patients with BTM had MCV values less than 72 fl in our study. MCV values of patients with IDA may be normal or low, depending on the IDA is acute or chronic. However, decreasing of MCV values as beta thalassemia minor patients are rare (12). In our study, the BTM group had the lowest average MCV value among the three groups. However, the BTM group's mean MCV value was only statistically significantly lower than the control group. The Mentzer index, used for differential diagnosis of microcytic anemia, is useful for screening tests. The index is calculated by dividing MCV by RBC count (13). Ehsani et al. (14) reported that the Mentzer index differentially diagnoses IDA with BTM with a sensitivity of 95%. In our study, Mentzer index values were greater than 13 for 14 of 20 children in the IDA group. The index values were lower than 13 for all 21 children in the BTM group. Per these results, the Mentzer index suggested the correct diagnosis for 90% of the patients.

Red blood cell distribution width (RDW) tends to be normal or slightly increased in beta thalassemia minor patients. Romero et al. (15) showed that RDW was a more sensitive indicator for differential diagnosis than MCV. In our study, RDW was higher in the IDA group than in the other two groups. The control group had the lowest RDW value.

The level of sTfR in circulation is an indicator of the total body concentration of TfR (16). Under normal circumstances, erythroid precursors are the main source of sTfR (17). The most important cause of increasing in sTfR is a lack of iron, during erythropoiesis (6). sTfR levels do not increase if iron stocks decrease but iron is present during erythropoiesis; the best indicator of this situation is a lower ferritin level. If iron deficiency continues and insufficient iron is supplied for erythropoiesis, sTfR levels begin to increase. This increase occurs before other indicators of iron deficiency (18). Chouliaras et al. (19) found that sTfR values were highest among IDA patients, followed by BTM, iron deficient, and control group individuals, in that order. In our study, sTfR levels were three times higher in the IDA group, and two times higher in the BTM group, compared to the control group.

In a study of the efficacy of using sTfR for differential diagnosis of IDA and BTM, sTfR levels were found to be higher in the IDA group. However, sTfR levels were not useful in determining of a differential diagnosis unless the patient's thalassemia trait was known (20). In our study, sTfR levels were high in the IDA group, but significant difference was not detected between the IDA group and BTM group.

In our study, the correlations between sTfR and hematologic and biochemical iron parameters were also investigated. There was no correlation between iron status and sTfR in the control group. sTfR showed a weak inverse correlation with Htc in the thalassemia group and a weak direct correlation with RDW and PLT values. No correlation was found between other hematological or biochemical values and sTfR. Danise et al. (21) found similar results in their study. While a meaningful negative correlation was observed between sTfR, serum ferritin and hemoglobin in healthy children in this study, no correlations were detected between sTfR, ferritin, hemoglobin, and MCV in the BTM group. sTfR levels in the iron deficiency anemia group were significantly and inversely correlated with Hb, Htc, MCV, MCH, and MCHC (p<0.001). However, there was not correlation for serum iron. These results are consistent with the literature (22). Jayaranee et al. (22) showed that there is a reverse correlation between sTfR and ferritin. Our study could not demonstrate this relationship.

According to the literature, the sTfR/ferritin ratio developed using the inverse relationship between sTfR and ferritin is a marker for determining the degree of iron deficiency (18). Lopez et al. (23) do not recommend using the sTfR/ferritin ratio because the ratio increases whether sTfR and sTfR/ ferritin levels are useful parameters for identifying IDA. Jayaranee S et al. found that the sTfR/ferritin ratio was high in the IDA group and low in the BTM group (22). The TfR/ferritin rate was higher in the control group than in the IDA and BTM groups, in accordance with the literature.

Kemna et al. (24) showed that the efficacy of using hepcidin levels for differential diagnosis decreased when thalassemia major and sTfR levels increased in the IDA group. They also detected a strong reverse correlation between hepcidin and sTfR and suggested that the correlation was related to increased erythropoietic activity and reduced hepcidin release (25). sTfR levels in the IDA and BTM groups were high in our study, but we did not observe decreased prohepcidin levels. No correlation between sTfR and prohepcidin was determined.

In a previous study, serum hepcidin levels of thalassemia major patients and urinary hepcidin levels of thalassemia patients were found to be low (25). Also, hepcidin levels were lower in patients with iron deficiency anemia (26). Prohepcidin levels were similar to those of the control group in our study. A study investigating a correlation between prohepcidin and hepcidin (24) found that hepcidin levels of the IDA and thalassemia major groups were not significantly different, and there was also no correlation between serum prohepcidin and hepcidin levels.

Conclusion

As a result, we determined that prohepcidin measurement does not reflect active hepcidin levels and that prohepcidin is not a useful clinical indicator because no difference in prohepcidin levels is observed between groups. Furthermore, no correlation was demonstrated between prohepcidin and other iron parameters. Instead, sTfR/ ferritin ratio may be useful for determining total body iron status of patients.

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Treatment of periodontal infrabony defects with beta-tricalcium phosphate and platelet-rich plasma in patients with advanced chronic periodontitis

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Abstract

Objectives: Regenerative periodontal surgery is an important adjunct to complex periodontal treatment. The goal of periodontal therapy is to prevent tissue destruction while achieving the regeneration of damaged tissues. The objective of this study was to evaluate the efficacy of β -tricalcium phosphate (β -TCP) and platelet-rich plasma (PRP) in regeneration of periodontal infrabony defects.

Materials and methods: Twenty six patients with advanced chronic periodontitis were treated with β - tricalcium phosphate (β -TCP) + PRP. Clinical and radiographic parameters, including: plaque index (PI), sulcus bleeding index (SBI) clinical attachment level (CAL), periodontal probing depth (PD), gingival recession (GR) and bone defect depth (a1), were evaluated at baseline, six months and year after surgery.

Results: Healing was uneventful in all patients. Mean PPD decreased from 6.44 mm to 3.17 mm (p<0.001), and to 2.97 mm (p<0.001) (6 months and 1 year respectively). Mean CAL changed from 7.99 mm to 5.12 mm (p<0.001), and to 5.0 mm (p<0.001) (6 months and 1 year respectively). Mean a1 decreased from 0.93 mm to 0.68 mm and to 0.57(6 months and 1 year respectively).

Conclusions: Within the limits of the present study, it can be concluded that at year after surgery significant PD reductions and CAL gains were found.

Clinical relevance: Combination of β -tricalcium phosphate and PRP can be used in successful treatment of vertical periodontal bone loss due to its contribution to clinical and radio-logical improvement of periodontal status.

Key words: Bone graft, platelet-rich plasma, β -*tri*calcium phosphate, periodontal bony defects, periodontal disease.

Introduction

Periodontal disease involves an inflammatory process leading to the destruction of periodontal tissues. These mechanisms are associated with osteolysis. Periodontal regenerative therapy, including surgical techniques and biomaterials used in the management of vertical bony defects, promote predictable regeneration of lost hard tissues. Alloplastic biomaterials, as β -tricalcium phosphate $(\beta$ -TCP) have been advocated to enhance bone formation. β -TCP might have a significant effect on bone formation. Presence of microparticles (size 500-1000 µm) creates a specific porous structure of this biomaterial. These features enable progressive angiogenesis and vascularization as well as influence resorption and cell transferring inside the bone defects. β-TCP is a biomaterial of short-term stability [1]. Developed surface of grains enables resorption both through hydrolysis and phagocytosis [2].

Clinical observations, according to Artzi et al. [3]. showed accelerated resorption of β -TCP in relation to osteogenesis, which leads to smaller volume of bone in comparison to the amount of used biomaterial, it has been noticed that, after 12 months, about 23-28% of nonresorbed material remains in the defect. Two year observation of Kozakiewicz et al. [4] proved of human model that architecture of newly formed bone and structure of nonresorbed grains in bone defects treated with β -TCP had similar structure to normal bone. In recent years, the combination of osteoconductive biomaterials with Platetet Rich Plasma (PRP) has been used in sur-

gical treatment of vertical bone defects. Polypeptide growth factors are bioactive mediators existing physiologically in tissues and are responsible for migration, proliferation, differentiation and metabolism of the wound. The cell response to the specific growth factor is determined by the presence of a suitable receptor localised in the cell membrane [5]. Sandro et al. [6] suggest that use of the combination of β-TCP with PRP might contribute to induction of periodontal tissues regeneration by PRP and osteoconductive influence of β -TCP. Other authors have reported that efficacy of the adjunct of platelet concentrates depends on the amount and concentration of platelets growth factors [7]. In addition, PRP, as an autogenic material, eliminates the risk of transferring infection or induction of immunological response. The main objectives of the study were: firstly, defining to what extent the biomaterial in combination with PRP influences regeneration of periodontal bone defects; secondly, assessment the parameters of periodontal status after surgery.

Materials and Methods

Patient selection for regenerative procedures

The study was conducted among 26 healthy individuals with chronic advanced periodontitis (24 females and 2 males). Non-surgical therapy was accomplished prior to surgery in all patients. After obtaining the informed consent to the proposed treatment plan, the instruction of oral hygiene was performed. Only those who maintained good oral hygiene were qualified for the surgery (PI: 0.4-0.6). The sites with vertical bone defects equal or deeper than 5 mm were chosen on the basis of periapical radiographs. The age span was 21-62 years (mean age 46.8). Among ninety periodontal bone defects subjected to treatment: seventy-four occurred in the maxilla (twenty-four incisors, fifteen canines, twenty premolars and fifteen molars) and sixteen occurred in the mandible (one incisor, three canines, five premolars and seven molars). This group consisted of twenty-five three-wall and sixty-five two-wall bone defects.

Radiological evaluation of the alveolar bone loss

The radiological evaluation was performed using right angle technique using Digora PCT MIN- RAY[®] (Soredex®, Tuusula, Finland) (parameters: 60kV,8mA, 0,6s) [8,9,10]. Digital periapical radiographs were evaluated on the Digora 2.5 for Windows software (Soredex®, Tuusula, Finland) (Figure 1):

- a) loss of alveolar bone as the total depth of defect (that is the distance a from 2 mm below CEJ to the bottom of bone defect)
- b) supralveolar loss of bone depending on the alveolar crest level a2
- c) intrabony depth of bone defect as a1
- d) width of bone defect h.



Figure 1. The scheme of the radiological measurements

Assessment of periodontal status

The clinical assessment included: Plaque Index – (PI) [11], Sulcus Bleeding Index- (SBI) Mühlemann and Son's [12] and Clinical Attachment Level (CAL), Periodontal Pocket Depth (PD), Gingival Recession (GR) and Mobility of Tooth (MT) (Periotest[®] Medizintechnik Gulden, Germany) were assessed in patients. Periodontal measurements were obtained using the Williams probe (Hu-Friedy[®], USA), which is marked in millimeters at the following distances from the probe tip: 1, 2, 3, 5, 7, 8, 9 and 10 millimeters.

Surgical procedures and application of biomaterial

Directly before the procedure 8.5 ml of blood from the patient's cubital vein was drawn into the

container CPDA (Citrate Phosphate Dextroseadenine) solution. PRP was obtained from the patient's blood using a special set of probes (PRP-Kit, Curasan AG, Kleinostheim, Germany) with centrifuge machine MPW-221/MPW-223 (Curasan AG, Kleinostheim, Germany). Full - thickness flaps were raised to maintain the greatest amount of gingival tissue. All of the bone defects were accessed using papilla preservation flaps, as described by Cortellini and Tonetti [13]. During removal of granulation tissue and exposing bone defect, some blood was taken from the operative site. The blood was combined with Cerasorb[®] and with PRP in glass container. This mixture was put into intrabony defect with the use of plastic applicator. The full-thickness flap was coronally positioned and closed with simple interrupted sutures (5/0, Premilene®, Braun, Germany). Patients were given the following postoperative recommendations: amoxicillin (500 mg three times a day for 7 days), mouth rinse containing 0.2% solution of chlorhexidine twice a day. The follow-up period

was 12 months. The examinations of the operated sites were performed after 3, 6, and 12 months including measurement of periodontal parameters and indicators mentioned in the methodology.

Statistical analysis

The obtained data was subjected to statistical analysis using Statistica 6.0 (StatSoft Inc., Tulsa, USA). Asymptotic normality of estimated parameters was investigated via Shapiro-Wilk test. In the presence of normal distribution of parameters the T-student test for paired samples was performed to compare the mean values in the groups. When the distribution of parameters was not normal, Wilcoxon matched-pairs test was used. Results that yield p-value<0.05 were considered statistically significant.

Results

Periodontal status and oral hygiene

The investigation showed that patients had maintained good oral hygiene. Mean values of PI

| Table 1. Mean values of clinical and radiological | al paramet | ters before an | d 3, 6 an | d 12 months | after sur |
|---|------------|----------------|-----------|-------------|-----------|
| gery with Cerasorb $^{\mathbb{R}}$ + PRP. | | | | | |

| Parameters Number of investigated periodontal pockets | baseline | 3 months after surgery 90 | 6 months after surgery 90 | 12 months after surgery 90 |
|---|----------|------------------------------|------------------------------|-------------------------------|
| P.I. | 0.65 | 0.51 | 0.41 | 0.33 |
| SBI | | 0.94 | 0.36 | 0.13 |
| CAL (mm) | 7.99 | 5.29 (+34.0%) | 5.12 (+35.7%) | 5.0 (+36.9%) |
| PPD (mm) | 6.44 | 3.39 (+46.7%) | 3.17 (+49.9%) | 2.97 (+52.9%) |
| GR (mm) | 1.81 | 2.19 (+26.9%) | 2.16 (+22.9%) | 2.22 (+23.4%) |
| МТ | 19.92 | 16.51 (20.2%) | 14.36 (31.9%) | 12.37 (38.8%) |
| al (mm) | 0.93 | m.d. | 0.68 (26.89%) | 0.57 (38.71%) |
| h (mm) | 0.80 | m.d. | 0.85 (6.25%) | 0.72 (10%) |
| a (mm) | 5.74 | m.d. | 5.02 (12.54%) | 5.00 (12.89%) |
| a2 (mm) | 4.81 | m.d. | 4.34 (9.78%) | 4.42 (8.11%) |

m.d. - missing data, PI - Plaque Index, SBI - Sulcus Bleeding Index, CAL - Clinical Attachment Level/Loss, PPD - Periodontal Pocket Depth GR - Gingival Recession, MT - Mobility of Tooth, section a - entire size of defect, section al - bonedefect depth (vertical bone defect), section a2 - the size of alveolar bone defect (horizontal defect), section h - the width ofthe bone defect, determined by the height of triangle ABC before treatment were on the level of 0.6 and, after 12 months, decreased to 0.3. The value of Sulcus Bleeding Index (SBI) after 12 months decreased from 1.51 to about 0.1 (Table 1). The regeneration or reparation of CAL has been proven by their significant decrease observed during all stages of investigation (after 12 months the CAL decreased to about 3 mm, Figure 2). The reduction of periodontal pocket depth (PD) is also a desired effect of regenerative procedures. In this investigation after 12 months following surgery it was noted that PD was decreased by almost 50% (from 6.44 mm to 2.97 mm) (Table 1, Figure 3). After surgical treatment there was significant decrease in tooth mobility (39% reduction). Mean Periotest® values were: 19.92 before and 12.37 after treatment. The mean value of gingival recession increased by 0.4mm (23%) after 12 months following surgery (Table 1).



Figure 2. Mean value of clinical attachment level gain (CAL)



Figure 3. Mean value of probing pocket depth reduction (PPD)

The sites with vertical bone defects equal or deeper than five millimeters were chosen on the basis of dental periapical radiographs. Among ninety periodontal bone defects subjected to treatment: seventy-four occurred in the maxilla (twenty-four incisors, fifteen canines, twenty premolars and fifteen molars) and sixteen occurred in the mandible (one incisor, three canines, five premolars and seven molars). This group consisted of twenty-five threewall and sixty-five two-wall bone defects.

Radiographic measurements of bone defects

Radiographs revealed changes in vertical and horizontal dimension of bone defects (Table 1, Figures 4, 5, 6, 7). After six and twelve months following surgery significant decrease of infrabony defects accounting for 27% and 39% in vertical measure and to about 6% and 10% horizontal measure was observed.



Figure 4. Mean value of bone defect depth - vertical bone defect (a1)



Figure 5. Mean value of the width of the bone defect (h)

Supralveolar loss of bone after 6 months significantly decreased from 4.81mm to 4.34 mm that is by almost 9.78% and after 12 months its value decreased to 4.42 mm that is by only 8.1% of rebuilding the alveolar crest (Figure 7). The mean value of gingival recession increased by 0.4mm (23%) after 12 months following surgery (Table 1).



Figure 6. Mean value of entire size of defect (a)



Figure 7. Mean value of the size of alveolar bone defect - horizontal defects (a2)

Discussion

The goal of regenerative therapy is achieving rebuilding of periodontal tissues which are functionally and structurally identical with lost tissues as restitutio ad integrum. It is impossible to confirm the regeneration of periodontal tissues through clinical examination, thus the histological evaluation is required [14]. However, clinical decrease of PD might be the effect of either regeneration of connective attachment, or reparation during creation of long junctional epithelium. In our study, after six months of observation, a statistically significant CAL gain by by 2.87 mm (from 7.99 mm to 5.12 mm) was noted, as well as reduction of PD by 3.23 mm (from 6.4 mm to 3.17 mm). After the same period of observation other authors [15] noted after performing GTR slightly worse results (CAL gain and PD reduction about 2.6 mm). In our study the values of these parameters were further improved, and after 12 months they reached statistically significant CAL gain by 2.99 mm and reduction of PD by 3.43 mm (from 6.4 mm to 2.97 mm). Similar results (CAL gain 2.7 mm and reduction of PD by 3.7 mm) were noted by other authors [16] after treatment of vertical bone defects using GTR with Gore-Tex membrane and Emdogain preparation (CAL gain of 2.8 mm and reduction in PD of 3.5 mm). Table 2 presented improvement of clinical and radiological parameters after application of different methods used in periodontal regeneration.

The results obtained in this research are similar to those obtained by Yassibag-Berkman et al. [17]. Similar observations were made by Harnack et al. [18] who used for treatment of bone defects β -TCP alone and in combination with PRP. The authors claimed that adding PRP to β-TCP in comparison to β -TCP alone did not lead to a statistically significant CAL gain and PD reduction after six months following the surgery. In their research the median of CAL gain after six months in the group of PRP + β -TCP amounted to 0.28 mm and in the group of β -TCP alone 0.13 mm. The median of reduction of PD 0.8 mm and 0.4mm accordingly (Table 2). Study results presented by other authors [19] indicate that adding DFDBA to PRP did not significantly influence improvement of clinical parameters compared with results obtained with the use of PRP alone. Similarly, Döri et al. [20] claimed that adding PRP to the combination of β -TCP and e-PTFE membrane did not lead to a statistically significant higher CAL gain nor to greater reduction of PD at the end of 12-month period of observation. It was noted that in the group of PRP + β -TCP + e-PTFE CAL gain amounted to 4.1 \pm 0.7 mm and in the group of β -TCP + e-PTFE to 3.9±0.9 mm. Reduction of PD was shaped at the level of 5.8 ± 0.6 mm and 5.4 ± 0.7 mm accordingly. Between the fourth and sixth week after surgical treatment the authors observed slight denudation of part of e-PTFE membrane in seven cases of treatment with the use of PRP and nine cases without in. In order to prevent bacterial infection a chlorhexidine gel and mouth wash were prescribed until the moment of membrane removal. The research results of other authors [21] confirm that PRP autologous rich platelet concentrate may reduce membrane uncovering after surgery and accelerate an increase of bone density. However, financial burden of this procedure and patients' discomfort is too excessive in comparison to anticipated positive effects. In their research with the use of PRP and hydroxyapatite with membrane

| Table 2. | Juxtaposi | tion of carried out clinical and radiol | ogical stuales after application affe | tern menous of periouoniai regener | nonn |
|--------------------------------|----------------------------|---|---|---|---|
| Study | Follow-up (months) | Gain in clinical attachment level (mean ± SD in mm) | Reduction in probing pocket depth (mean ± SD in mm) | Gingival recession (mm) | Bone fill (mm) |
| Shih-Yun | , | GTR (Atrisorb): 2.40 ± 1.17 | GTR (Atrisorb): 2.50 ± 1.35 | GTR (Atrisorb): 000 ± 1.63 | GTR (Atrisorb): 0.33 ± 1.89 |
| Wu et al. ¹³ | 9 | GTR (Bio-Mend): 2.60 ± 1.26 Access flap: 1.80 ± 0.92 | GTR (Bio-Mend): 2.60 ± 1.08 Access flap: 2.20 ± 1.14 | GTR (Bio-Mend): 0.00 ± 0.67 Access flap: 0.40 ± 0.52 | GTR (Bio-Mend): 2.57 ± 1.64 Access flap: 1.05 ± 0.77 |
| Crea | 5 | GTR (Gore-Tex): 2.7 ± 1.2 | GTR (Gore-Tex): 3.7 ± 1.2 | GTR (Gore-Tex): 1.0 | GTR (Gore-Tex): 2.9 |
| et al. ¹⁴ | 17 | Emdogain: 2.8 ± 1.3 | Emdogain: 3.5 ± 1.3 | Emdogain: 0.6 | Emdogain: 2.3 |
| | , | , | β-TCP + PRP + GTR(BioGide): 3.4 | | |
| Yassibag- | 9 | m.d. | β-TCP + PRP: 2.9 β-TCP: 3.4 | m.d. | m.d. |
| Berkman | | β -TCP + PRP + GTR(BioGide): 2.5 | β -TCP + PRP + GTR(BioGide): 4.0 | | |
| CI 411. | 12 | β -TCP + PRP: 2.1 B TCD: 2.4 | β-TCP + PRP: 3.6 β TCD: 4-1 | m.d. | m.d. |
| | | Median in mure | D-LCF. 4.1 Modion in mure | | |
| Harnack | 9 | PRP $+$ 6-TCP: 0.28 | PRP $+$ B-TCP: 0.8 | m.d. | $PRP + \beta$ -TCP: 1.1 mm |
| et al. ¹⁶ |) | β-TCP: 0.13 | β-TCP: 0.4 | | β-TCP: 1.3 mm |
| | | $PRP - 3.08 \pm 0.95$ | $PRP \cdot 3.92 \pm 1.1$ | PRP: 0.33 ± 1.44 buccal | |
| Markou et al. ¹⁷ | 9 | PRP + DFDBA: 3.08 ± 1.17 | PRP + DFDBA: 3.75 ± 1.49 | 0.83 ± 0.94 lingual PRP + DFDBA: 0.83 ± 1.12 buccal | m.d. |
| | | | | 1.17 ± 1.27 lingual | |
| Döri | <u>;</u> | PRP + β -TCP + e-PTFE: 4.1 ± 0.7 | PRP + β -TCP + e-PTFE: 5.8 ± 0.6 | $PRP + \beta$ -TCP + e-PTFE: 1.4 ± 0.8 | -c \$ |
| et al. ¹⁸ | 17 | β -TCP + e-PTFE: 3.9 ± 0.9 | β -TCP + e-PTFE: 5.4 ± 0.7 | β -TCP + e-PTFE: 1.5 ± 0.7 | III.Q. |
| | ŝ | β-TCP+GTR(Resolut XT)+APC: 5.6±1.5 | β-TCP+GTR(Resolut XT)+APC: 6.3±1.2 | B-TCP+GTR(Resolut XT)+APC: 1.0±1.3 | m.d. |
| |) | β -TCP + GTR(Resolut XT): 5.4 ± 2.3 | β -TCP + GTR(Resolut XT): 6.2 ± 1.7 | β -TCP + GTR(Resolut XT): 1.0 ± 1.3 | |
| Christgau | 9 | β-TCP+GTR(Resolut XT)+APC: 5.3±1.8 | β-TCP+GTR(Resolut XT)+APC: 6.4±1.5 | β-TCP+GTR(Resolut XT)+APC: 1.0±1.3 | h d |
| et al. ¹⁹ | þ | β -TCP + GTR(Resolut XT): 5.3 ± 2.0 | β -TCP + GTR(Resolut XT): 6.1 ± 1.3 | β -TCP + GTR(Resolut XT): 0.9 ± 1.3 | |
| | 12 | β-TCP+GTR(Resolut XT)+APC: 5.0±1.5 β-TCP + GTR(Resolut XT)· 5.2 ± 1.6 | β-TCP+GTR(Resolut XT)+APC: 6.3±1.2 β-TCP+GTR(Resolut XT): 6.0±1.1 | B-TCP+GTR(Resolut XT)+APC: 1.3±1.3 B-TCP+GTR(Resolut XT) 1 0 ± 1 2 | m.d. |
| Yamamiya | ; | $HCP + PRP + HA: 3.9 \pm 1.6$ | $HCP + PRP + HA: 4.8 \pm 1.1$ | HCP + PRP + HA: 0.9 ± 1.5 | $HCP + PRP + HA: 4.9 \pm 1.2$ |
| et al. ²⁰ | 17 | $PRP + HA: 2.7 \pm 1.3$ | $PRP + HA: 4.3 \pm 1.1$ | $PRP + HA: 1.7 \pm 1.3$ | $PRP + HA: 3.2 \pm 1.1$ |
| | " | β -TCP: 2.6 ± 12 | β -TCP: 2.9 ± 1.6 | β -TCP: 0.52 ± 0.71 | m d |
| Tavakumar | ٠ | rhPDGF-BB + β -TCP: 3.2 ± 1.0 | rhPDGF-BB + β -TCP: 3.2 ± 1.6 | rhPDGF-BB + β -TCP: 0.44 ± 0.77 | |
| et al. ²¹ | | β -TCP: 2.8 \pm 0.9 | β-TCP: 3.2 ± 1.6 | B-TCP: 0.54 ± 0.73 | β-TCP: 2.8±1.2(47.5%±19.8) |
| | D | rhPDGF-BB + β -TCP: 3.7 ± 1.0 | rhPDGF-BB + β -TCP: 4.3 ± 0.9 | rhPDGF-BB + β -TCP: 0.44 ± 0.77 | $(65.6\% \pm 21.7)$ |
| m.d. – miss | ing data, β . | -TCP - beta tricalcium phosphate, PRP - F | latelet Rich Plasma, GTR – Guided Tissue | ? Regeneration, e-PTFE – non-bioresorbab | ble expanded polytetrafluo- |
| roethylene – deminera | membrane, Ilized freeze | APC – autologous platelet concentrate, rh. e-dried bone allograft, HA – hydroxyapatite | PDGF-BB - recombinant human platelet-d , HCP – human cultured periosteum | lerived growth factor, APC - autologous pl | atelet concentrate, DFDBA |

Yamamiya et al. [22] noted CAL gain by 2.7±1.3 mm and PD reduction by 4.3±1.1 mm whilst adding of HCP (human cultured periosteum) led to CAL gain by 3.9±1.6 mm and PPD reduction by 4.8±1.1 mm. According to the authors in the layers of this membrane there are osteogenic cells which increase the regenerative potential of periodontal tissues. The study of Jayakumar et al. [23] provide interesting observations. Authors used for treatment of vertical bone defects purified recombinant human platelet-derived growth factor (rhP-DGF-BB) in concentration of 0.3 mg/ml rhPDGF-BB+ β -TCP 3.7±1.0 mm and 4.4±0.9 mm, while in group of β -TCP alone 3.7±1.0 mm and 3.2±1.6 mm accordingly.

However, flap surgery might lead to the root cementum denudation. In our study with the use of Cerasorb[®] + PRP after 6 months following surgery we noted an increase in the mean value of gingival recession from 1.81 mm to 2.16 mm (by 0.35 mm), whereas after 12 month to 2.22 mm (by 0.41 mm). Obtained results were similar to those of Jayakumar et al. [23] which after 6 months following surgery amounted to 0.44±0.8 mm for rh-PDGE-BB+ β -TCP biomaterials versus 0.54±0.73 mm for β -TCP alone. Other authors [20] noted much greater values of gingival recession after 12 months: in the group with PRP + β -TCP + ePTFE membrane by 1.44 mm and in the group without PRP by 1.5 mm.

The design of infrabony defect has its influence on the surgical treatment effectiveness [13]. In our study after 6 and 12 months the x-ray screen showed a decrease in infrabony defects in vertical dimension in relation to the measurements before treatment by 27% and 39% accordingly. In horizontal dimension the width of bone defects decreased after six and twelve months by about 6% and 10% accordingly. Supracrestal bone loss was significantly reduced after 6 months from 4.81 mm to 4.34 mm and after 12 months to 4.42 mm, which constituted 9.78% and 8.1% of alveolar crest height rebuilding accordingly. Figures 8 and 9 present radiographs of the bone defect on teeth 44 before and 12 months after surgery Cerasorb[®] + PRP.



Figure 8. Radiographic appearance of osseous defect in tooth 44, radioghraph obtained before surgery



Figure 9. Radiographic appearance of osseous defect in tooth 44, radiograph obtained 12 months after surgery

Yassibag-Berkman et al. observed [17] the greatest regeneration of hard tissues was noted in the group of β -TCP+PRP+GTR comparing to other groups after 6 months. However, after 9 and 12 months filling of hard tissues in the group β -TCP alone gradually increased to the same level as in the group of β -TCP+PRP+GTR, whereas β -TCP+PRP still showed much less filling with bone tissue. Radiographic examination showed partially non-resorbable granules of β -TCP after twelve months, granules of β -TCP+PRP much fewer granules were observed. On the basis of these observations the authors suggested that PRP can accelerate β -TCP resorption by increasing

inflammatory reaction through macrophages. Results of Yassibag-Berkman's [17] studies have proven PRP did not improve regeneration of periodontal tissues in human model. Well documented results after using GTR method in combination with biomaterial minimize the possibility of presenting a positive influence of PRP. During interpretation of PRP studies it is worth to remember that the exact mechanism of PRP's influence on regenerative processes of periodontal tissues has not been defined yet [20].

Few authors perform a second surgery to control formed bone tissue. Harnack et al. [18] performed a re-entry surgery after 6 months and claimed that in the group treated with β -TCP alone the depth of infrabony defect amounted to 3.3 mm (median) before surgery while during re-entry it was 1.6 mm. In the group of β -TCP+PRP they noted 3.5 mm (median) before treatment and 2.4 mm during re-entry surgery whereas addition of PRP did not increase the amount of new hard tissues. In the research of Jayakumar et al. [23] after six months noted filling bone defects by 65.6± 21.7%.

Conclusion

Further investigations are needed to estimate the PRP influence on increasing the regenerative potential of periodontal tissues. Defining of adequate concentration of individual growth factors included in PRP might be helpful. Currently, there are a few ways of selecting PRP from patients' blood, and various methods provide different platelet and growth factors' concentrations [24]. Periodontal regenerative surgery appears to be a very important part of complex treatment of periodontal diseases. Preceded by professional nonsurgical therapy and always followed by with meticulous oral hygiene instruction. Moreover, patients should cooperate and be aware of the necessity of specific maintenance therapy in such chronic and infectious condition as periodontal disease.

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Red blood cell transfusions and survival of sickle cell patients

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Abstract

Background: We tried to understand whether or not there is an association between increased amount of red blood cell (RBC) transfusions and prolonged survival of sickle cell diseases (SCDs) patients in long term.

Methods: All patients with SCDs were enrolled into the study. Cases with RBC transfusions of less than 10 units in their lives were put into the first, cases with transfusions of 10 units or higher were put into the second, and cases with transfusions of 50 units or higher were put into the third groups, and the groups were compared in between.

Results: The study included 284 patients (139 females). Mean ages of the three groups were similar (28.8, 28.8, and 29.6, respectively, p>0.05between all). Interestingly, there was a progressive and significant increase according to the male ratio from the first towards the third groups (37.7%, 49.5%, 66.2%, p<0.05 between all). Although the prevalences of cirrhosis, coronary heart disease, and exitus were similar in the three groups (p>0.05 between all), there were progressive and significant increases according to mean painful crises per year, smoking habit, digital clubbing, chronic obstructive pulmonary disease, leg ulcers, stroke, chronic renal disease, and pulmonary hypertension from the first towards the third groups (p < 0.05, nearly in all steps).

Conclusion: Although RBC transfusions may be a life-saving approach during severe painful crises, particularly crises with end-organ failures, and although the higher units of transfused RBCs may show clinical severity, it does not correlate with a prolonged survival in SCDs cases in long term.

Key words: Red blood cell transfusion, sickle cell diseases, atherosclerosis.

Introduction

Systemic atherosclerosis induced aging may be the major pathology of the human being. Atherosclerosis is an irreversible process initiating at birth, and is probably accelerated by many factors. Some of the accelerating factors are collected under the heading of metabolic syndrome including smoking, overweight and obesity, dyslipidemia, white coat hypertension and hypertension (HT), and insulin resistance and diabetes mellitus (DM) for the development of eventual consequences such as coronary heart disease (CHD), chronic obstructive pulmonary disease (COPD), cirrhosis, chronic renal disease (CRD), peripheric artery disease, blindness, and stroke (1-6). Sickle cell diseases (SCDs) are a prototype of accelerated systemic atherosclerotic process (7-9). They are characterized by sickle-shaped erythrocytes which is caused by homozygous inheritance of the hemoglobin S (Hb S). Glutamic acid is replaced with valine, as a less polar amino acid in the sixth position of beta chain of the Hb S. Presence of the less polar amino acid promotes polymerisation of the Hb S. So Hb S causes erythrocytes to change their normal elastic and biconcave disc shaped structures to a hard and sickle shaped bodies. The rigidity of erythrocytes is the central pathology of the disease. The sickling process is probably present in whole life, but it is exaggerated during various stressful conditions. 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. The sickled cells induced chronic endothelial damage causes secondary tissue ischemia and infactions, even in the absence of an obvious vascular occlusion. Finally, life expectancy of the SCDs cases is decreased by 25

to 30 years (10). We tried to understand whether or not there is an association between increased amount of red blood cell (RBC) transfusions and prolonged survival of the SCDs in long term in the present study.

Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and March 2013. All patients with SCDs were enrolled into the study. SCDs are diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography (HPLC). Their medical histories including numbers of painful crises per year, units of transfused RBC in their lives, regular alcohol consumption, smoking habit, leg ulcers, and stroke were learnt. Cases with a history of three pack-year were accepted as smokers. A check up procedure including serum iron, total iron binding capacity, serum ferritin, serum creatinine value on three occasions, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, an electrocardiography, an abdominal ultrasonography, a Doppler ultrasonography to evaluate the portal blood flow, an endoscopy to detect esophageal varices just in suspected cases, and a computed tomography of the brain was performed. Cases with acute painful crises or any other inflammatory event were treated at first, and then the spirometric pulmonary function tests to diagnose COPD, the Doppler echocardiography to measure the systolic blood pressure (BP) of pulmonary artery, renal and hepatic function tests, and measurement of serum ferritin level were performed on the silent phase. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% (11). Systolic BP of the pulmonary artery of 40 mmHg or higher during the silent phase is accepted as pulmonary hypertension (12). CRD is diagnosed with a permanently elevated serum creatinine level which is 1.3 mg/dL or higher on the silent phase. Cases with renal transplantation were put into the CRD group. Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, esophageal varices, and ascites without any histologic procedure in the absence of indication. Digital clubbing is diagnosed by determining of the ratio of distal phalangeal diameter to interphalangeal diameter which is required to be higher than 1.0, and with the presence of Swamroth sign (13,14). Associated thalassemias are detected by serum iron, total iron binding capacity, serum ferritin, and the hemoglobin electrophoresis performed via HPLC. A stress electrocardiography was performed in cases with an abnormal electrocardiography and/or history of angina pectoris. A coronary angiography was obtained just for the stress electrocardiography positive cases. So CHD was diagnosed, angiographically. Eventually, cases with RBC transfusions of less than 10 units in their lives were put into the first, cases with transfusions of 10 units or higher were put into the second, and cases with transfusions of 50 units or higher were put into the third groups, and the three groups were compared in between. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 284 patients with SCDs (145 males and 139 females). There were 90 cases (31.6%) in the first, 105 cases (36.9%) in the second, and 89 cases (31.3%) in the third groups without any significant difference in distribution (p>0.05). There was not a significant difference according to the prevalence of associated thalassemias between the three groups, either (Table 1). Mean ages of the three groups were similar, too (28.8, 28.8, and 29.6 years, respectively, p > 0.05 between all). The mean units of transfused RBCs were 4.1, 21.3, and 95.9, respectively. There were 13 cases (4.5%) without any RBC transfusion in their lives, and the mean age of them was 27.9 ± 6.6 (17-39) years, again. Interestingly, there was a progressive and significant increase according to the male ratio from the first towards the third groups (37.7%, 49.5%, 66.2%, p < 0.05 between all). There was not any patient with regular alcohol consumption among the study cases. Although the prevalences of cirrhosis, CHD, and exitus were similar in the three groups (p>0.05between all), there were progressive and significant increases according to mean painful crises per year, smoking habit, digital clubbing, COPD, leg ulcers,

stroke, CRD, and pulmonary hypertension from the first towards the third groups (p<0.05, nearly in all steps) (Table 2). Mean ages of the mortal cases were 25.7 ± 6.3 (19-39) and 31.0 ± 10.6 (19-45) years in males and females, respectively (p>0.05). On the other hand, five of the CRD cases were on hemodialysis, and one with renal transplantation. Although antiHCV was positive in two of the cirrhotic cases, HCV RNA was detected as negative by polymerase chain reaction in both. Histological diagnosis of cirrhosis was required in none of the study cases.

Discussion

Painful crises are the most disabling and nearly pathognomonic symptoms of the SCDs. For example, only 10.2% of the study cases (21 females and eight males) have not had any crisis in their lives in the present study. Although these crises may not be life threatening, directly (15), infections are the most common precipitating factors of them. SCDs cases are immunocompromised due to a variety of reasons including a functional and anatomic asplenism, chronic endothelial damage induced end-organ insufficiencies, a permanent

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

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

*Red blood cell

†Difference between the first and third groups ‡Nonsignificant (p>0.05)

| 100102. Dienie celi pullentis with absociated absorael | . Sickle cell patients with associated disorder |
|--|---|
|--|---|

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

*Red blood cell

†Difference between the first and third groups

 $\ddagger Nonsignificant (p>0.05)$

§Chronic obstructive pulmonary disease

¶Chronic renal disease

**Coronary heart disease

inflammatory process all over the body, recurrent hospitalizations, frequent transfusions, and usual invasive procedures. Because of the relative immunodeficiency, even simple infections may progress to severe sepsis in a short period of time. Therefore multiorgan failures are not unusual during acute painful crises in such cases. So the risk of mortality is significantly higher during the crises, and repeated RBC transfusions may be necessary to provide the survival. In such severe situations, RBC transfusions may provide adequate tissue oxygenation and immunity, so prevent intractable pain, dissemination of infections, and end-organ failures. On the other hand, pain is the result of a poorly understood interaction between sickled cells, endothelial cells, leukocytes, and platelets, yet. The adverse actions of platelets and neutrophils on endothelium are of particular interest in SCDs cases. For instance, leukocytosis even at silent period was an independent predictor of the severity of the SCDs (16), and it was associated with the risk of stroke (17). On the other hand, leukocytosis and thrombocytosis are acute phase reactants that are nearly present in all SCDs patients even during the silent periods. They indicate presence of a permanent inflammatory process initiating at birth. The continuous inflammatory process alone causes an additional accelerated atherosclerotic process and a relative weight loss in the SCDs cases (18). 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 intractable pains. Hospital admissions for acute painful crises typically last for 4-10 days, but the time varies greatly. Because of the severity of pain, narcotic analgesics are usually required to control them (19), but according to our practice, simple RBC transfusions may be highly significant during severe painful crises, both to relieve pain and prevent sudden deaths secondary to multiorgan failures developed on the chronic background of SCDs.

Because of the repeated infarctions and subsequent fibrosis during early years of life, an asplenism develops with the decreased antibody production, prevented opsonization, and reticuloendothelial dysfunction in adults. 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 (16). In another study, the peak incidence of death occured between 1 and 3 years of age in children, and the deaths were predominantly caused by pneumococcal sepsis in patients less than 20 years of age (20). As also observed by us in the present study that SCDs cases, even those who appear relatively fit, are susceptible to sepsis induced multiorgan failure and sudden death during acute painful crises due to the generalized immunosuppression.

Because of the chronic endothelial damage, SCDs affect all organ systems where the blood goes in the body (21-22). Even there were patients with severe vision or hearing losses among our study cases. Aplastic crises, sequestration crises, hemolytic crises, acute chest syndrome, avascular necrosis of the femoral and humeral heads, priapism and infarction of the penis, osteomyelitis, acute papillary necrosis of kidneys, chronic renal failure, pulmonary hypertension, bone marrow necrosis induced dactilitis in children, chronic leg ulcers around ankles, hemiplegia, and cranial nerve palsies are some of the other presentation types of the SCDs. Eventually, the mean survivals were 42 and 48 years for males and females in the literature (13), whereas the mean ages of mortal cases were 25.7 and 31.0 years in males and females in the present study, respectively. The great differences between the survival may be secondary to the initiation of hydroxyurea treatment in infancy in the developed countries. On the other hand, the significantly higher male ratio, painful crises per year, digital clubbing, COPD, leg ulcers, stroke, CRD, and pulmonary hypertension of the cases with RBC transfusions of 50 units or higher, the lower mean ages of the male patients with mortality, and the lower ratio of male cases without any painful crisis in their lives (5.5% of males, 15.1% of females) should be searched with further studies. 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 (18). On the other hand, as an opposite finding

to some other reports (23-24), the mean heights were nearly similar in the SCDs with the controls in the above study (18). Probably due to the significantly lower body weight and BMI, mean values of the low density lipoprotein cholesterol, alanine aminotransferase, and systolic and diastolic BPs were also significantly lower in the SCDs cases (18), which can be explained by definition of the metabolic syndrome (25-26).

As a conclusion, although RBC transfusions may be a life-saving approach during severe painful crises, particularly crises with end-organ failures, and although the higher units of transfused RBCs may show clinical severity, it does not correlate with a prolonged survival in SCDs patients in long term.

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The inhibitory effect of propolis on osteoclast maturation

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Abstract

Background: This study investigated the inhibitory effect of Azerbaijan propolis on osteoclast maturation.

Methods: In this in vitro experimental study, 3 Swiss Webster mice with the age of 4-6 weeks were sacrificed and osteoclast cells were obtained from their bone marrow. Animals bone marrow cells were placed on the culture medium and the wells were exposed to Azerbaijan propolis of 1 μ l and 10 μ l concentrations or control. The specimens were stained for tartrate-resistant acid phosphatase(TRAP)-activity to identify osteoclasts using a light microscope with x20 magnification. The cells number was classified in three groups of mononuclear, multinuclear and giant and the data were subjected to the analysis of variance (ANO-VA) and tukey post hoc tests.

Results: In the first day the number of mononuclear cells in the propolis groups was significantly higher than control group, while the value was significantly higher in the 10 μ l concentration compared to 1 μ l concentration. Furthermore, the number of multinuclear and giant cells was significantly higher in the control group compared to the propolis groups, while more number of these cells was observed in 1 μ l concentration than 10 μ l concentration.

Conclusion: Azerbaijan propolis inhibits osteoclastogenesis and osteoclasts activation on in vitro condition.

Key words: Inhibitory effect, Osteoclast maturation, Propolis.

Introduction

Dental traumatic injuries are considered as common problems in dentistry (1). Specially avulsion injuries that are identified through the displacement of the whole tooth from the alveolar socket and their successful replacement totally depends on immediate practical treatment (2,3,4,5). On the other hand, two main factors of treatment failures consist of inflammatory root resorption and replacement resorption. Following replantation, the attachment apparatus of the root is injured and in some cases, cellular necrosis and macrophage activation in alveolar bone is inevitable (6). Nowadays there are serious debates about the best and most appropriate treatment options having the least toxic effects in achieving a successful tooth replantation treatment (2). Among the materials currently in use, calcium hydroxide inhibits inflammatory root resorption in replanted teeth. However it is temporary and no efficient measures are usually taken to prevent consequent resorption (7). Propolis is produced as a natural material by honey bees in different geographic areas and its composition depends on the variety of plants in each area. Its components have anti-inflammatory and analgesic effects (8,9). On the other hand, some laboratory researches have shown an increasing capacity of pulp healing by propolis in the healing process of pulp tissues along with the formation of dentinal bridge (10,11,12). Also in a recent study it has been established that systemic use of propolis may accelerate formation of new bones at the expanded suture in rats (13). According to the importance of having an appropriate material for successful treatment in replantation and considering the potent anti-inflammatory properties of propolis, the aim of this in vitro study was determined as the inhibitory effect of propolis on osteoclast maturation.

Methods and Materials

The propolis used in this research was a brown sample from Azerbaijan. The early form of this

material was in solid form which was sliced into small pieces prior to the onset of procedure. Then, 0.5 gram of propolis was added to an Erlenmeyer containing 30 cc of 67% ethanol solution(6). The Erlenmeyer was located in a shaker for 24 hours and finally, pieces which were not dissolved, removed by filter paper. Once more, 100 cc of ethanol was added to the contents of the Erlenmey which was kept in a shaker for another 24 hours. After repeating this course for 3 times, the resulted solution was placed in a vacuum distillation apparatus and eventually alcoholic propolis extract was achieved. In the present study, osteoclasts were obtained from the bone marrow of 4-6 weeks old Swiss Webster mice which were ultimately sacrificed. This study was approved by ethics committee of Shahid Beheshti University of Medical Science. Mice bone marrow was extracted by excision of both bone ends, flushed with the use of a 25 gauge needle in "α-modified Minimum Essential Medium"(sigma) culture medium and added to a 10% fetal bovin serum (aMEM D10). Animal bone marrow cells were washed twice with α MEM. Then the nucleated cells were counted and the survival rate of these cells was estimated over 95%. In the next stage, 9×105 cells were located in each cm2 in the wells of three culture plates, each containing 24 places (A,B,C) which contained aMEM D10 and 1,25-dihydroxyvitamine D3 (calcitriol)(6). Vitamin D3 provokes the differentiation of pre-osteoclast cells into multinuclear osteoclasts. On this basis, wells were allocated to control groups (C), propolis 1μ l on the first day (T1), propolis 1μ l on the fifth day (T5), propolis 10 µl on the first day (T1), propolis 10 μ l on the fifth day (T5).

The cultures were located in an incubator with 98% humidity and 37°C and 5% CO2, and the cultured sample was replaced every two days. After 7 days, the culture of cells was fixed with 2% paraformaldehyde solution and in order to be identifiable, the samples were colored by tartrate resistant acid phosphatase (TRAP) which was supplied from Sigma company. TRAP positive cells were counted using a light microscope with x20 magnification, averaged, and classified in three groups of mononuclear (1 nucleus), multinuclear (2-10 nucleus) and giant cells (more than 10 nucleus). Microscopic investigations were carried out by a pathologist who had no idea about the type of gro-

ups. Moreover, the effect of different propolis concentrations on the number of multinuclear TRAP positive cells in the fifth day and after fixation of cells, was studied under light microscope and with the same magnification.

The data were evaluated with the analysis of variance (ANOVA) and Tukey post hoc tests.

Results

Based on the results obtained from this research, the number of mononuclear cells was 250, the number of multinuclear cells was 200 and the number of giant cells was 10. The results obtained for 1 μ l concentration of propolis showed that at the time T1 (first day) the number of mononuclear cells were 360, the number of multinuclear cells were 150 and the number of giant cells were 5. With this concentration, the number of multinuclear cells in the fifth day was 197.

The results obtained for 10 μ l concentration of propolis showed that at the time T1 (first day) the number of mononuclear cells were 600, the



Figure 1. The average number of mononuclear, multinuclear and giant cells number in different concentrations: Day 1

Number of multinuclear cells were 120 and no giant cells were identified in this concentration. (Figure 1). Besides, the number of multinuclear

cells in the fifth day was 195. Therefore according to the results of variance analysis test (ANOVA) there were significant differences between control group and two different concentrations of propolis regarding the number of mononuclear cells in the first day (ρ <0.0001). Moreover the result of tukey test comparison revealed that on this day the number of mononuclear cells in both propolis concentrations were obviously and significantly higher than the specimen of control group $(\rho < 0.0001)$ and the number of mononuclear cells in the 10µl concentration of propolis were significantly more than that in 1µl concentration of propolis (ρ <0.0001). Also on the first day the number of multinuclear cells in different concentrations of propolis was estimated to be significantly less than the specimens of control group ($\rho < 0.0001$). Based on the results of tukey comparative tests the number of multinuclear cells in the control group were significantly more than both concentrations of propolis and their number in 1µl concentration was significantly more than those in 10µl concentration (ρ <0.0001). On the first day the results were studied regarding the number of giant cells and the number of these cells in control group were significantly higher than their number in both concentrations of propolis (ρ <0.0001). On the fifth day the number of multinuclear cells in the 1µl concentration was hardly more than their number in 10µl concentration. In comparison with control group, no significant difference was observed in 1µl and 10µl concentrations. Samples of histologic observations in different groups are presented in figure 2 to figure 6.



Figure 2. Bone marrow cells culture in the presence of calcitriol



Figure 3. Bone marrow cells culture in the presence of $1\mu l$ propolis



Figure 4. Bone marrow cells culture in the presence of $10 \mu l$ propolis



Figure 5. Giant cell in culture medium of bone marrow



Figure 6. Osteoblasts in culture medium of bone marrow

Discussions

Based on the results of the present study, the number of multinucleated and giant cells in the presence of 1µl and 10µl propolis was lower than the control group. Also the number of mononuclear cells in both concentrations of propolis was obviously more than the control group. In addition the number of multinucleated cells in 10µl propolis was lower than 1µl propolis (120 versus 150). Propolis in both concentrations of 1 µl and 10 µl and only one day after exposure was able to prevent the maturation of mononuclear pre-osteoclast cells and also their conversion to multinuclear and giant cells to some extent. These effects were more frequent in 10 µl concentrations in comparison to 1µl concentrations. Nevertheless after 5 days exposure of samples to propolis, both concentrations showed similar behavior regarding the number of multinuclear cells (197 in 1µl propolis and 195 in 10µl propolis). As a matter of fact it can be concluded that it does not have considerable effect on the reduction of multinuclear cells. Exposure of the samples to both concentrations of Azerbaijan propolis could reduces the number of multinuclear and giant TRAP+ cells after 1 day, and it had an inhibitory effect on osteoclastogenesis. This inhibitory effect is dependent to the dose of propolis and it is targeting the early phase of osteoclastogenesis.

Pileggi demonstrated that propolis has the ability to inhibit osteoclast formation (reducing the number of TRAP+ multinuclear cells) and maturation(production of actin rings)(6). Also a research by Ang et al. (2009) showed that CAPE as one of the constituents of propolis has the capability to prevent osteoclast formation by suppressing Nuclear Factor Kapa B (NF- κ B) activity, implied by RANKL. Along with that the effects of CAPE on osteoclastogenesis fundamentally occurs in the early stages of cellular differentiation (14).

It seems that propolis has the capability to prevent some of the paths leading active osteoclasts maturation. The results of a previous research showed that propolis has exerted anti – inflammatory effects by preventing the cyclo-oxygenase pathway and eicosanoid synthesis after cornea injuries (15).

Osteoclastogenesis phenomenon requires activation of NF- κ B by a product of COX pathway, i.e. PGE2. The probable justification which seems to be logical in this regard is that this path might have been the primary target of propolis, although more research is required for the assessment of this idea (16).

Root resorption is the main concern of clinicians about traumatized teeth (17). Up to now, calcium hydroxide has been the preferred medication for these cases.

Kristerson and Andreasen showed that using calcium hydroxide in canals following teeth replantation, resulted in the occurrence of considerable replacement resorption in comparison to the cases where gutta-percha was used in the canals or pulps were extirpated and no material was used for filling the canals (18). Besides, Hovland and Dumshar in a preliminary research found that using calcium hydroxide was not effective in prevention of consequent resorption after avulsion injuries (19).

On the other hand Al-Shaher showed that the amount of toxicity of calcium hydroxide was 10 times more than propolis, such that in concentrations of 0.4 mg, more than 75% of PDL fibroblasts and 90% of Pulp fibroblasts were destroyed(9).

Recently in a research using propolis, it was reported that, there is not any difference, regarding resorption, in using fluoride or propolis on the surface of roots in the replanted teeth of rats (20).

On this basis, the results of the present study show that propolis has the ability to prevent ostenoclastogenesis and activate osteoclasts on in vitro condition.

It seems that more research is necessary to study the effects of other concentrations of propo-

lis in reducing the rate of root resorption by means of other techniques and at the same time the effects of different chemical compositions of propolis should be evaluated.

Conclusion

The results of the present research regarding the inhibitory effect of Azerbaijan propolis on maturation of osteoclasts, has shown that exposing the specimens to both concentrations of propolis has considerably reduced the number of TRAP positive multinuclear and giant cells after only one day exposure. This effect is also related to the applicable dose of propolis and higher concentrations of the material shows better results. Also the inhibitory action of propolis, had targeted the early phase of osteoclasts formation. In sum, propolis has the ability to inhibit osteoclastogenesis and to activate osteoclasts on in vitro conditions.

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Polish pregnant women and their oral health: Epidemiological analysis from north-east part of Poland

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Abstract

The purpose of this study was to assess the dental health of pregnant women living in rural and urban settings in north-east part of Poland.

Methods: The study group comprised 228 pregnant women aged between 17 and 41 years. The research was a part of the monitoring carried out in Poland. Oral examination was carried out according to WHO recommendations. Caries intensity expressed as the mean DMF and CPI index were evaluated in examined women. The clinical examination helped to establish dental treatment needs of surveyed women.

Results: Patients from rural settings constituted 26.31%, and from urban areas 73.69%. Pregnant women living in urban areas had fewer cavities, extracted teeth and more fillings compared to women from rural areas. Dental treatment needs of women from urban residence were smaller compared to women from rural residence. Needs for pulp treatment, teeth extraction and advanced periodontal disease requiring specialist treatment occurred more often in women living in rural areas.

Conclusions: Place of residence in rural areas affects the number of untreated carious lesions and the number of extracted teeth in women of childbearing age.

Oral health prevention programs should target pregnant women living in rural settings.

Key words: Pregnant women, DMF, epidemiology, rural and urban residence.

Introduction

Dental care of pregnant women should include health education, the prevention and treatment strategies. The implementation of proper hygiene habits of the mother will not only reduce the risk of dental caries and periodontal disease in the pregnant women, but will also affect the future child (1-5). Mothers-to-be ought to take care of a reasonable diet, rich in vegetables, fruits and dairy products, calcium and vitamins and limit the intake of sweets (acido-, cariogenic) (6,7). Women are focused on the health of future children, so they try to change the eating habits during pregnancy, but those from the urban settings do so more often (64.08%) compared to women from rural areas (56.31%) (8,9).

Dental caries is an infectious disease. Teeth with caries in mothers promote transmission of pathogenic bacteria responsible for the development of this disease to child via close contacts between mother and child usually associated with the performance of daily personal hygiene and feeding (7,10). Therefore, the awareness and behavior of the mothers-to-be, the maintenance of good oral hygiene and the caries treatment are the most important factors that may reduce the risk of tooth decay in their offspring. Authors emphasize that the oral health of the mother is mostly affected by non-medical factors such as education, place of residence and economic status (11-13).

Changes in the female's body occurring during pregnancy are reflected in the oral cavity. Apart from dental caries disorders of the secretion of saliva, lower pH and lower buffer capacity occur in pregnant women (14). Frequent vomiting in early pregnancy and heartburn may lead to the formation of teeth erosion localized especially on the palatal surfaces of the upper front teeth. They are also the reason for tooth hypersensitivity. Moreover, the elevated concentration of steroid hormones in pregnancy both in blood and saliva may play a role in the severity of inflammation in periodontal tissues. The largest increase of gingivitis in pregnancy is observed between 14 and 30 weeks of gestation. The factors that modify the inflammatory response in the gums during pregnancy apart from the changes in hormone levels include also specific changes in bacterial flora and the changes in the immune response of pregnant women. Specific changes in dental plaque depend on the growth of Prevotella intermedia. In vitro studies suggest that the bacteria of this species may use hormones, estrogen and progesterone, as growth factors (as a substitute for vitamin K) (15-17). Other study suggests an association between periodontal disease and adverse pregnancy outcomes, including preterm birth or low birth weight, gestational diabetes, preeclampsia (11).

The period of pregnancy is a heavy burden for the woman's mentality (13). The best time for dental treatment is the second trimester of pregnancy.

The purpose of this study was the clinical evaluation of dental health of pregnant women living in rural and urban settings in Podlaskie province, Poland.

Methods

The study included in total 228 pregnant women aged between 17 and 41 years (mean age 29.95). The research was a part of the national monitoring under the National Oral Health Surveillance. The places were drawn according to strict rules developed by the World Health Organization (WHO) for the similar epidemiological studies which took place throughout the country. The selection of the sample was held in each case on the basis of a twotier draw. In the first stage the Chief Professional Officer for the Health Surveillance designated one district of a rural and an urban area, respectively, from north-east part of Poland. In the second stage, regional consultant designated gynaecological practices that pregnant women attended for recalls. The number of respondents was limited by the survey assumptions, and not to exceed 250 so the response rate was 91,2%.

The study started after the approval from the Ethics Committee and written consents of pregnant women were collected. All women were examined by an experienced, calibrated examiner. The study was conducted according to the recommendations of the WHO (18). Data from the clinical examination was entered into individual epidemiological study forms. Oral examination was carried out in a dental office, in artificial light, using a mirror, en explorer and a periodontal probe. Caries intensity expressed by the mean DMFT was evaluated including: the number of teeth with active caries (D), teeth extracted due to caries (M) and filled teeth (F), dental treatment index (DTI) and treatment needs. The periodontal status was assessed using the Community Periodontal Index (CPI) according to the criteria: 0 -sound, 1 -bleeding from the gingival pocket during probing, 2 - presence of dental plaque or subgingival calculus, 3 - depth of periodontal pockets of 3.5-5.5 mm, 4 – depth of periodontal pockets above 5.5 mm, X – sextant excluded (teeth extracted).

Statistical analysis

Clinical results were statistically analyzed using the IBM[®] SPSS[®] Statistics 20.0. The analyzed parameters are described by an arithmetical mean (x), standard deviation (SD), minimum and maximum values and percentages (%). In assessing the differences between two independent groups for the examined characteristics the nonparametric Mann-Whitney test and the Pearson's χ^2 test for independence were used. Statistical results with pvalue lower than 0.05 were considered significant.

Results

Women from the rural environment constituted 26.31%, and 73.69% from urban residence. The majority of patients (82.1%) were in the third trimester of pregnancy and only 2,2% in the first. In both groups women with higher education dominated, however, such education was one and a half times more frequent in women from urban settings. The lowest number of subjects declared primary or vocational education; 25% in rural areas and 7.74% in urban settings, respectively (Table 1). Most women were expected their first child. However the second child was expected almost two times more often by women living in rural than in urban areas. Women in rural settings made the decision to have a third child more often than women from the urban areas whereas the fourth pregnancy concerned only women in rural areas. This relationship was statistically significant (Table 2).
| Education $n(0/)$ | Place of | | |
|-----------------------|-----------|------------|---------|
| Education, II (76) | rural | urban | |
| Elementary/vocational | 15(25.0) | 13 (7.7) | |
| Secondary | 20 (33.3) | 36 (21.4) | p<0.001 |
| Higher | 25 (41.7) | 119 (70.8) | |

Table 1. Pregnant women from urban and rural residence depending on education level

* Pearson's χ^2 test for independence

Table 2. Distribution of urveyed population depending on number of pregnancies

| No of programming $n(9/)$ | Place of | ** * | | | |
|-----------------------------|-----------|-------------|-------|--|--|
| No. of pregnancies, if (76) | rural | urban | P | | |
| first | 29(48.33) | 135(80.36) | | | |
| second | 20(33.33) | 29(17.26) | 0.000 | | |
| third | 7(11.67) | 4(2.38) | 0.000 | | |
| fourth and more | 4(6.67) | 0(00) | | | |

* Pearson's χ^2 test for independence

Table 3. The DMFT and its components depending on place of residence

| | Mean | SD | Minimum | 1. Quartile | Median | 3. Quartile | Maximum | |
|-------|-------|------|---------|-------------|--------|-------------|---------|---------|
| D | | | | | | | | |
| rural | 4.17 | 4.92 | 0 | 0 | 3 | 6 | 18 | p=0.005 |
| urban | 1.92 | 2.66 | 0 | 0 | 1 | 3 | 16 | |
| Μ | | | | | | | | |
| rural | 2.12 | 2.32 | 0 | 0 | 1 | 3 | 9 | p<0.001 |
| urban | 1.02 | 1.55 | 0 | 0 | 0 | 2 | 9 | |
| F | | | | | | | | |
| rural | 8.65 | 5.11 | 0 | 4.25 | 9 | 12 | 20 | p<0.001 |
| urban | 12.01 | 4.05 | 2 | 9 | 12 | 15 | 20 |] |
| DMF | | | | | | | | |
| rural | 14.93 | 5.14 | 3 | 12 | 15.5 | 18 | 25 | p=0.94 |
| urban | 14.95 | 3.91 | 2 | 13 | 15 | 18 | 25 | |
| TI | | | | | | | | |
| rural | 0.68 | 0.34 | 0 | 0.49 | 0.77 | 1 | 1 | p=0.002 |
| urban | 0.86 | 0.18 | 0.2 | 0.78 | 0.93 | 1 | 1 | |

rural n=60 urban n= 168 Mann-Whitney test DTI= Dental treatment Index

A comparison of the mean DMFT in examined pregnant women revealed that this value was only slightly higher in the group of women from rural than from urban areas. The differences were not statistically significant. The number of missing teeth and with cavities was significantly higher in women from rural areas. In contrast, women living in urban settings had a higher value of the F component, and this difference was statistically significant. Also statistically significant differences, occurred in the dental treatment index, in favor of women from urban areas (Table 3). Women from urban areas statistically significantly often had no treatment needs than women from rural areas (Table 4). The needs for pulp treatment and tooth extraction occurred especially in the group of women from rural areas, and these differences were statistically significant.

Women living in urban areas presented better periodontal condition than those from rural settings and concerned mostly sextants with CPI = 4, P=0.03, which reflected the most advanced periodontal disease where periodontal pockets were more than 5.5 mm deep, and with excluded sextants, P=0.004. Periodontal treatment needs of the sampled population concerned mostly the improvement of oral hygiene and the removal of dental deposits (Table 5).

| | Moon | <u>SD</u> | Minimum | 1 Quantila | Modian | 2 Quantila | Maximum | *** | |
|-------------------------------|---------|-----------|----------|-------------|---------|-------------|---------|----------|--|
| | wiean | 50 | winninum | 1. Quartile | wiedian | 5. Quartile | Maximum | <i>p</i> | |
| no needs | | | | | | | | | |
| rural | 26.55 | 5.63 | 13 | 23 | 28 | 31.75 | 32 | 0.015 | |
| urban | 28.88 | 3.50 | 16 | 27 | 30 | 32 | 32 | | |
| filling on 1 surface | | | | | | | | | |
| rural | 1.17 | 1.44 | 0 | 0 | 1 | 2 | 6 | 0.143 | |
| urban | 0.94 | 1.54 | 0 | 0 | 0 | 1 | 10 | | |
| filling on 2 or more surfaces | | | | | | | | | |
| rural | 1.57 | 2.28 | 0 | 0 | 1 | 2.75 | 9 | 0.054 | |
| urban | 0.89 | 1.52 | 0 | 0 | 0 | 1 | 11 | | |
| treatment of pulp diseas | ses and | filling | | | | | | | |
| rural | 0.50 | 1.16 | 0 | 0 | 0 | 1 | 7 | 0.000 | |
| urban | 0.08 | 0.31 | 0 | 0 | 0 | 0 | 2 | | |
| extraction | | | | | | | | | |
| rural | 0.93 | 2.25 | 0 | 0 | 0 | 1 | 11 | 0.000 | |
| urban | 0.04 | 0.22 | 0 | 0 | 0 | 0 | 2 | | |
| no data | | | | | | | | | |
| rural | 1.22 | 2.39 | 0 | 0 | 0 | 0.75 | 10 | 0.784 | |
| urban | 1.08 | 1.93 | 0 | 0 | 0 | 1 | 7 | | |

Table 4. Treatment needs of pregnant women depending on place of residence.

rural n=60 urban n= 168 Mann-Whitney test

Table 5. Periodontal status of pregnant women depending on place of residence

| | Mean | SD | Minimum | 1. Quartile | Median | 3. Quartile | Maximum | <i>p</i> * | |
|------------------------------|-----------|------|---------|-------------|--------|-------------|---------|------------|--|
| No. of sextant | s with CP | [=0 | | | | | I | | |
| rural | 4.30 | 1.89 | 0 | 3 | 5 | 6 | 6 | 0.174 | |
| urban | 4.68 | 1.68 | 0 | 4 | 5 | 6 | 6 | | |
| No. of sextants with CPI = 1 | | | | | | | | | |
| rural | 0.28 | 0.76 | 0 | 0 | 0 | 0 | 4 | 0.415 | |
| urban | 0.32 | 1.04 | 0 | 0 | 0 | 0 | 6 | | |
| No. of sextants with CPI = 2 | | | | | | | | | |
| rural | 0.17 | 0.49 | 0 | 0 | 0 | 0 | 3 | 0.070 | |
| urban | 0.26 | 0.48 | 0 | 0 | 0 | 0.75 | 3 | | |
| No. of sextant | s with CP | [=3 | | | | | | | |
| rural | 0.85 | 1.31 | 0 | 0 | 0 | 1 | 6 | 0.269 | |
| urban | 0.67 | 1.16 | 0 | 0 | 0 | 1 | 6 | | |
| No. of sextant | s with CP | [=4 | | | | | | | |
| rural | 0.13 | 0.47 | 0 | 0 | 0 | 0 | 3 | 0.031 | |
| urban | 0.05 | 0.35 | 0 | 0 | 0 | 0 | 3 | | |
| Excluded sext | ants | | | | | | | | |
| rural | 0.27 | 0.86 | 0 | 0 | 0 | 0 | 4 | 0.004 | |
| urban | 0.02 | 0.13 | 0 | 0 | 0 | 0 | 1 | | |

rural n=60 urban n= 168 Mann-Whitney test

Discussion

This was the first and so far the only nationwide study conducted in Poland provided with insight in oral health of pregnant women. Our results are difficult to compare with the nationwide surveys conducted in other countries because there the risk factors of oral disease often include socioeconomic conditions and living in immigrant neighborhoods. The social structure is also different from our reality because living in rural settings does not always result in poorer education and lower health.

Oral health in pregnant women from urban areas was better than from rural settings. Although the mean DMFT in patients of both groups was similar we observed differences among the D, M and F components between pregnant women living in urban and rural settings. Some authors acknowledge that the place of residence affects the number of teeth with caries (10,19). Almost all studies conducted in Poland claimed that the highest D component occurred in rural areas, slightly smaller in small towns, and the lowest in big cities. High values of carious teeth, reflect the state of dental care, eating habits, level of health-related awareness and long-term preventive treatment actions. Residents of rural areas had almost two times more carious cavities than those who lived in urban settings. In addition, the dental health of pregnant women is complemented by the mean number of fillings. Women in urban areas had more than 11 fillings whereas those living in rural areas had just over eight fillings, so the differences are statistically significant. In our study the F component is consistent with treatment index and better education of pregnant women from urban settings and was higher than the national average of 0.75 (20). We think that one of the reason for this situation might be disparities in education level among women and difficult access to dental practices for women from rural areas. Because in Poland dental practices both private and public are mostly located in the cities and still some procedures are limited. Another issue is fact that still some physicians and obstetricians believe that women lose teeth due to pregnancy (21).

As we can see the oral health status of pregnant women presented in a national survey and in our research is far from ideal. Apart from treating dental caries there is a strong need to cure pulpal infections and extract teeth especially in rural settings. Wojtyła et al (8), in their study on nutrition in pregnancy, suggested that educational activities in rural communities should be conducted. Rural residents have a poorer health than those living in urban settings, which may be also translated into the oral health (19).

The period of pregnancy has also an unquestionable impact on the periodontal status. According to our study, rural residents have more advanced periodontal diseases and greater periodontal treatment needs. A nationwide study revealed 28.9% of women with healthy periodontium, the lowest percentage was 17.2% and in our region healthy periodontium constituted 39.9% of women which was above the national level (20).

This study revealed the need to develop and implement a comprehensive program of oral health care for pregnant women, especially for those who live in rural areas. An essential part of such programs should be the education focused on the maintenance of general health (13).

Conclusion

Pregnant women from urban settings presented better oral health status.

Place of residence in rural areas affects the number of untreated carious lesions and the number of extracted teeth in women of childbearing age.

Oral health prevention programs should target pregnant women living in rural settings.

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Publication ratio of oral and poster presentations at annual meetings of Turkish neurosurgical society between 2005 and 2009

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Abstract

Objective: The aim of this study was to evaluate oral and poster presentations in the annual meetings of Turkish Neurosurgical Society (TNS) from 2005 to 2009, and their publication ratios in peer-reviewed international journals.

Methods: All oral and poster presentations of the annual meetings of the TNS within a five year period from 2005 to 2009 were evaluated. Publications related to these presentations have been searched in the PubMed search engine.

Results: There were a total of 2672 abstracts between 2005 and 2009, presented at annual meetings of the TNS. The number of published articles in peer-reviewed journals was 414 and the overall publication rate was 15.5%. Publication rate of oral presentations (24.6%) were found significantly higher (P<0.05) than poster presentations (12.3%).

Conclusion: This is the first study evaluating oral and poster presentations in these meetings of TNS from 2005 to 2009 and their publication ratios in peer-reviewed international journals.

The publication rate of the TNS meeting abstracts was found to be lower than that of publications ratios of meetings of Congress of Neurological Surgeons (CNS) and American Association of Neurological Surgeons (AANS). Causes of lower publication ratios need to be determined and improvement of these ratios should be encouraged.

Key words: Neurosurgery, presentation, oral, poster, publication rates, annual meetings.

Introduction

At annual meetings of clinical specialties, oral and poster presentations offer invaluable opportunities for dissemination of new research findings (18); however, scientific impact of presentations in clinical meetings is generally limited. Publication rate of abstracts in peer-reviewed journal may reflect the impact and scientific importance of the meetings at which the abstracts presented (4, 9). Sometimes these abstracts are considered as preliminary publications. However, the majority of the abstracts presented at scientific meetings would never be published in peer-reviewed journals. Therefore, it is not generally recommended in using the abstracts as bibliographic references, unless they have been published in peerreviewed journals (6, 8). For this reason, there is a need for motivation of investigators to publish their research in international journals after presentations in the clinical meetings (3, 6, 8, 9, 10).

In the literature there are not many studies evaluating the publication ratios of presentations of scientific meetings. By means of neurosurgery, we could find only one report from the United States of America in the English literature (6). In addition, by means of national meetings, there are only two reports from Turkey (11, 12). There is no study related to publication ratios of presentations in the congresses of neurosurgery in Turkey.

The aim of this study was to assess oral and poster presentations in the annual meetings of the Turkish Neurosurgical Society (TNS) from 2005 to 2009. We evaluated and compared publications of each meeting in peer-reviewed international journals. We also compared the differences and effects of the presentation type, both as a poster and as an oral presentation.

Materials and Methods

The meetings of TNS were held annually in April or May. Within a five year period from 2005 to 2009 all oral and poster presentations from these meetings of the TNS were evaluated. Abstracts were recruited from the official journal of TNS. All abstracts without any limitation (regarding subspecialty) were included in this study. A Pub Med database search was performed to determine whether or not the abstracts had been published in a peer-reviewed journal. Pub Med database search included the names of the authors, listed presentation titles, and the key words found in the title. Result was considered positive if the title of the paper, authorship, and eventual abstract in the published manuscript contained substantial similarities, as those published in the meeting proceedings. If the initial search did not hit any result, we then expanded the search to include the last name and first initial for every author listed. We then manually cross-referenced the key words from the title until a match was found or otherwise it was determined that there was no match. We recorded the following information: name of the journal in which the article was published, and number of months passed from the date of presentation at the meeting to the date of publication. There were various differences between presentations at the meetings and the corresponding published articles. In general, those differences were in the title of the research, the authors in the final article who were added or removed, change in the order of authorship, and change in the study of population.

Statistical Analysis

Data was presented as a number or a percentage. Pearson's chi-square test was used to compare publication ratios of the years of study. A p value of less than 0.05 was accepted as significant.

Results

A total of 2689 abstracts were presented at the TNS meetings between the years of 2005 and 2009. Of these 2689 abstracts, 431 (16.0 %) were published in peer-reviewed literature. Seventeen of these 2689 abstracts, were published before presentation at the TNS meeting, and thus were excluded from our analysis. Therefore, our study included 2672 abstracts that accounts for a publication ratio of 15.5%. Figure 1 presents total publication ratios of 2005, 2006, 2007, 2008, and 2009 years. The total publication ratio of 2005 year was significantly higher than those of 2006, 2007, 2008, and 2009 years (16.9%, 13%, 11.7%, and 13.1%, respectively vs. 22.9%; p<0.05). We found that the total publication ratios of 2006, 2007, 2008, and 2009 were comparable (p>0.05).



Figure 1. Total publication ratio of presentations in annual meetings of Turkish Neurosurgical Society between the years 2005 – 2009.

Furthermore, for all the years from 2005 to 2009, the publication ratios of oral presentations (33.1%, 26.6%, 17.6%, 23.3%, 23.2%, respectively) were found significantly higher than those of the poster presentations (19.1%, 13.6%, 10.8%, 8%, 10.3% respectively) (P<0.05) (Figure 2). Overall, the publication ratio of oral presentation was twice times higher than that of poster presentation from 2005 to 2009 years (24.6% vs. 12.3%, respectively; P<0.05).



Figure 2. Publication ratio of oral and poster presentations in annual meetings of Turkish Neurosurgical Society between the years 2005 - 2009.

| 8 | | | |
|-------|-------------------|---------------------|--------------------|
| Year | Oral presentation | Poster presentation | Total presentation |
| 2005 | 84.8% | 70.8% | 76.3% |
| 2006 | 78.9% | 87.5% | 84.0% |
| 2007 | 92.8% | 97.3% | 95.4% |
| 2008 | 96.5% | 87.1% | 91.7% |
| 2009 | 100% | 100% | 100% |
| Total | 89.4% | 86.5% | 87.7% |

Table 1. The period between publications and presentations after the annual meetings of Turkish Neurosurgical Society

Table 2. The top 10 journals that published articles from the meetings of Turkish Neurosurgical Society

| Rank | Journal | Percentage |
|------|---|------------|
| 1 | Journals of Turkish Neurosurgical Society | 20.8% |
| 2 | Journal of Clinical Neuroscience | 8.7% |
| 3 | Acta Neurochirurgica (Wien) | 5.6% |
| 4 | Surgical Neurology | 5.3% |
| 5 | Child's Nervous System | 4.3% |
| 6 | Turkish Journal of Trauma and Emergency Surgery | 3.6% |
| 7 | Neurosurgery | 3.4% |
| 8 | Journal of Neurosurgery Spine | 3.1% |
| 9 | Journal of Neurosurgery | 2.7% |
| 10 | Spine | 2.2% |

When looking at the period between publications and presentations, 87.7% of all eventual publications were published within 36 months after the presentation at the meeting (Table 1).

After presentation at the meeting the publication rate for oral presentations was found to be 89.4%, and for poster presentations it was found to be 86.5% within 36 months.

During the period of study, an average of 534 abstracts per year was presented, leading to an average of 83 publications per meeting that appeared in peer-reviewed journals.

Table 2 lists the top 10 journals that published articles from these meetings. Duplicate publications were not seen. Twenty percent of the published abstracts were eventually published in Journals of Turkish Neurosurgical Society. There are two journals of The TNS. These journals are named Turk Norosirurji Dergisi and Turkish Neurosurgery. In the list of the top 10 journals, the Journal of Clinical Neuroscience was the second and Acta Neurochirurgica was the third most frequent journals in which these studies were published.

Discussion

In this study, we evaluated the publication rates of the annual meetings of the presentations of TNS by exploring the PubMed database to see whether or not they had turned into an article. Our methodology incorporated a cyclical process which includes positing a question, researching previously discovered information, formulating a hypothesis, testing the hypothesis, interpreting the results, and then communicating the results.

The strength and prestige of a scientific meeting may be assessed by the subsequent publication of articles from the presented abstracts. Studies have indicated that the overall publication rate of submitted manuscripts from various medical specialties ranges from 30% to 81% (1-10, 13).

Between the years of 2005 and 2009, 2672 abstracts were presented at the TNS meetings, 414 (15.5%) of those were published in peer-reviewed literature. The total publication ratio of the year 2005 was found higher compared to those years of 2006-2009. There is only one study related to the publication of presentation of neurosurgery meetings (6). In this report the publication rate of the presentations at the annual meetings of the Con-

gress of Neurological Surgeons and the American Association of Neurological surgeons for a 3- year period from 2003 to 2005 was found to be as 32.48% (6). In another study, from 1991 to 1993 the publication rate of the presentations at the meetings of Scoliosis Research Society (SRS) and International Society for the Study of the Lumbar Spine (ISSLS), and North American Spine Society (NASS) from 1990-1992 was reported as 43.5% (13). The individual rates of these three societies were similar; 47%, 45%, and 40% for SRS, ISSLS, and NASS respectively. In addition, these ratios are very high compared to our results. In the meetings from 1993 to 2007, the publication rates of The Society of Cardiothoracic Surgery in Great Britain and Ireland were found to be between 30% and 81.3% (9). In this study, the publication rate of presentations at TNS meetings from 2005 to 2009 was found to be relatively insufficient. When compared to these reports, the overall publication rate of the presentations at the TNS meetings from the period 2005 to 2009 seems to be lower. Although most investigators argue for the lack of their time as a reason for not publishing findings presented in abstracts, many other factors may predispose to low publication. This involves the rate/time limitation, small sample size, non-experimental study design, negative results, lack of interest in pursuing further research, difficulty in writing in a foreign language; and the fact that publications necessitates more demanding work than presentations (3, 4, 6, 7, 13).

From a national and local perspective, there is limited number of studies evaluating this important issue related to annual meetings of Turkish clinical societies. There are two studies related to the publication ratios of presentations of annual meetings of dermatology and general surgery specialties. The publication ratio after the dermatology meeting was reported as 13.2%, whereas this ratio was reported as 5.7 % for general surgery (11, 12).

Oral presentations had a higher publication rate than that of poster presentations. This may be a result of the higher quality of such presentations. The reason why oral presentations are more published than posters may be because the referees might put in more effort in their selections as giving the best publication possibilities to oral presentations. During the study period, within 36 months, after presentation at the meeting 87.7% of all presentations were published. Within 36 months the publication rate for oral presentations was found to be 89.4%, and for poster presentations it was found to be 86.5%. When the published articles were taken into account, there was no difference in time in publication between oral and poster presentations.

This study was subject to limitations. Firstly, although it is widespread and despite extensive searching, the publication search may be incomplete due to the use of only one database as Pub Med. Another limiting factor in this study was the length of the time between the date of the meeting and the publication that can be insufficient especially for the years of 2008 and 2009.

TNS annual meetings serve private and academic Turkish Neurosurgeons. Additionally, these meetings also provide researchers with a medium from which they can receive critical feedback as they prepare their study for publication in a peerreviewed journal that is the ultimate goal of any research project. Publication in a peer-reviewed, MEDLINE indexed journal represents formal acceptance and validation of a study's findings and ensures broad dissemination of the information.

We believe that the publication ratios of presentations at the TNS meetings should be improved by encouraging authors and supporting research projects. In addition, submitted presentations should be carefully filtered for acceptance with respect to their scientific quality, since high quality researches promise higher subsequent publication potential.

The final target of meeting presentations should be the publication in a peer-reviewed journal. If publication rates increase, this contributes to the total quality of that medical specialty. Unpublished research does not provide a high enough chance for the dissemination of the evidence they present.

The evaluation of oral and poster presentations in the annual meetings of TNS from 2005 to 2009 and their publication ratios in peer-reviewed international journals provides an important base for improving scientific quality of meetings of TNS. There is a need to determine causes of lower publication ratios of oral and poster presentation.

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The application of 256-slice CT with low dose in neonates with hypoxic-ischemic encephalopathy

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Abstract

Objective: The purpose of this study was to assess the clinical value of 256-slice spiral CT with low dose in the neonate brain.

Methods: 150 newborns suspected to have Neonatal hypoxic-ischemic encephalopathy (HIE) and scheduled for a brain CT scan were randomly assigned into three equal size groups; standard dose group (120kV, 250mAs), low dose group 1 (120kV, 150mAs), and low dose group 2 (120kV, 50mAs). All other acquisition parameters were the same, in all groups. The CT dose index (CTDI), dose length product (DLP) and the image noise were compared between the three groups. The image quality was evaluated by blinded readers.

Results: (1) The DLP of the low dose group 2 was 19.3% of the conventional dose group without a significant difference (P>0.05); (2) The image noise of the low dose group was larger than the conventional dose group with a significant difference (P<0.01).

Conclusions: Low dose scanning is feasible in the screening of HIE in neonates, and beneficial to protect the newborn against unnecessary radiation damage.

Key words: Low-dose cranial CT, HIE, 256-slice spiral CT, Brain CT scan.

Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) may result from diffuse hypoxic-ischemic brain injury. It is one of the most common causes of cerebral palsy and other permanent neurological defecits in children. Therefore, a brain CT scan is commonly used in the screening and diagnosis of HIE. However, newborns are far more radiosensitive than adults and suffer from potentially more serious injury, thus reducing the radiation doses in neonatal CT scans is an important area of research in contemporary imaging technology [1]. A 256-slice CT scanner has the fastest rotation speed, and the lowest scattering lines as well as a novel detector, which can guarantee lower radiation doses during examination. The purpose of this study was to assess the overall image quality and clinical value of 256-slice spiral CT with low dose in the neonate brain suspected with HIE.

Materials and Methods

Clinical data

From March 2011 to March 2012, we selected 150 newborns in our hospital. There were 88 male and 62 female subjects; including 95 full-term and 55 premature cases. Among them, the average gestational age was 28 to 40 weeks, and birth weight ranged from 1,650 to 3,150g. 111 babies were born via natural labor and the remaining 39 cases were delivered by ceasarean section.

Methods

The CT scanner used in this study was a Philips Brilliance 256 slice spiral CT (Philips Medical System, City, Country). This scanner automatically displays the dose weighted index (CTDI) and dose-length product (DLP) during scanning.

Acqusition parameters

All patients were randomly divided into three groups according to the radiation dose. The standard dose group with 120Kv, 250mAs; the low dose group 1 with 120Kv, 150mAs and the low dose group 2 with 120Kv, 50mAs. The slice thickness was 5mm, and the interlayer spacing was also 5mm. The axis scan mode and other reconstruction parameters were the same in all groups. The newborn was in a supine position and a full brain scan was done.

The CTDI and DLP were recorded from the scanner display As DLP is related to the scanning range, CTDI and DLP in conventional dose and low-dose of the same scan length were also recor-

ded. We also measured the CT values in the left basal ganglia by drawing the region of interest (ROI).

Image quality assessment

The images were blinded assessed by two experienced physicians. Evaluation criteria: score 3 had no image artifacts, sharp edges of the skull, good contrast between the gray and white matter, clear ventricle edge, and obvious lesions; score 2 had some image artifacts, lower signal to noise. However, the reduced image quality did not affect the overall diagnosis; Score 1 images had more noise and the lesions were not clearly delineated. This could make an accurate diagnosis difficult and confusing.

The CTDI, DLP, signal noise and image quality were compared among the three groups. We used SPSS 13.0 software to perform the statistical analyses. P <0.05 was considered statistically significant.

Results

Image Quality Rating

The quality score was 2.55 ± 0.29 , 2.25 ± 0.41 and 2.05 ± 0.74 and in the conventional dose group, low dose group 1, and low dose group 2, respectively. There was no statistical difference in image quality rating between the three groups (P> 0.05).

Noise

The signal noise of the ROI was 1.78 ± 0.42 HU, 1.95 ± 0.35 HU and 2.36 ± 0.49 HU and in the conventional dose group, low-dose group 1 and low-dose group 2, respectively. The measured noise level showed a statistical significant difference between the groups (P < 0.05).

Radiation dose

The DLP was 311.6 mGy, 109.7 mGy and 60.2 mGy and in the conventional dose group, low-dose group 1, and low-dose group 2, respectively. The ratio of the low-dose group 1 to the conventional dose group was 35.2%, and the ratio of the low dose group 2 to the conventional dose group was 19.3%. With a decrease in the radiation dose, the DLP value also declined. However, this did not affect the image quality. Although the image noise was relatively high in the 50mAs group (low dose group 2), intracranial structures and lesions were clearly delineated. Both window and level settings can be adjusted to generate a diagnostic image. In summary, a 256-slice CT scan using a lower radiation dose can be used to safely screen the neonatal brain without a decrease in the overall image quality.



Figure 1. Standard dose



Figure 2. Low dose group 1



Figure 3. Low dose group 2

| Group | KV | mAs | CTDI(mGy*cm) | DLP (mGy) | SD(HU) | Image quality |
|------------------|-----|-----|--------------|-----------|-----------------|---------------|
| Standard dose | 120 | 250 | 30.4 | 311.6 | 1.78 ± 0.42 | 2.55±0.29 |
| Low dose group 1 | 120 | 150 | 24.8 | 109.7 | 1.95±0.35 | 2.25±0.41 |
| Low dose group 2 | 120 | 50 | 6.2 | 60.2 | 2.36±0.49 | 2.05±0.74 |

Table 1. Parameters and results of CT scan

Discussion

Low-dose CT scanning is being used in a variety of clinical applications. In the neonatal skull and brain the tissue density is not as high as in adults. Also, the successful application of lowdose CT scanning requires good natural contrast between the skull, brain tissue and the ventricular system. In the present study, we reduced the radiation dose in multi-slice spiral CT scanning of the newborn brain without compromising the image quality. The appropriate dose will ensure that the image quality has adequate contrast between normal brain structures and lesions in order to make a correct clinical diagnosis.

Multislice spiral CT (MDCT) can achieve a larger volume of data acquisition, which broadens the applications of CT and improves the diagnostic level. 256 slice CT scanning adopts the conversion technology of high-speed data, and reduces the electronic noise in the image chain. This improves the signal-to-noise ratio, and ultimately may reduce the X-ray dose. It can also improve the image quality to a certain extent, by compensating for a decrease in image quality due to the increased noise which may reduce the radiation damage to patient. Consequently, 256-slice spiral CT can be used in the study of newborns suspected with HIE. Our results showed that there was no diagnostic difference in the image quality between the low dose group and the conventional dose (P > 0.05).

The higher the applied X-ray dose used during a CT scan is, the greater the likelihood is for radiation damage. Previous studies have shown that after receiving the same radiation dose, the risk of brain tumors and leukemia in children is much higher than in adults, and the younger child is the greater the risk (2). Due to the specificity of neonatal physiology, children will be exposed to larger doses, and potentially develop more radiation damage than adults under the same scanning conditions. Therefore, the parameters of CT examination for children and newborns should be adjusted accordingly. Also, a smaller field of view and collimator should be used (3). Reducing the radiation dose in CT scanning is the main goal in protecting the neonetal brain. In the current study, the DLP in the lowest dose group was about 80% lower than in the conventional dose group (60.2 versus 311.g mGy). The CT images were all of diagnostic quality and the newborn brain was protected.

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Safety and effectiveness of fibrin glue for the closure of bladder incisions in rats

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Abstract

Background: We analyzed the safety and effectiveness of fibrin glue for the closure of bladder incisions in rats.

Materials and Methods: Each of the 30 Wistar-Albino rats was assigned one of three equal groups. A 5 mm long bladder incision was performed on all of the animals. In Group I, the incision was closed by 5/0 dexon. In Group II, after approximation by 2 - 3 transient sutures, fibrin glue was applied to the edges for five minutes. Then, the transient sutures were removed. For Group III, after the application of fibrin glue to the edges, the incision was approximated by 5/0 dexon stay sutures. The presence of extravasations was sought by cystography. And post-mortem histological examinations for fibrosis, oedema, inflammation and bleeding were recorded.

Results: Oedema and inflammation were significantly different among the three groups of animals (P = 0.001). Oedema was significantly higher in Group I than in Group II and Group III (P < 0.001, P = 0,017). Inflammation was significantly more prominent in Group I than in Group II and Group III and Group III (P = 0.001, P = 0,046). Fibrosis and bleeding were not significantly different. Cystography examination revealed minor changes.

Conclusions: Our findings confirmed that the fibrin glue application significantly reduces oedema and inflammation at the healing site of the bladder incision. Despite the 2.22 times increased relative risk of death with fibrin glue only repair, the application of fibrin glue together with suturing may be useful for better healing and preventing urinoma formation.

Key Words: Fibrin glue, suture, bladder, incision, experimental.

Introduction

The usage of fibrin glue is widely investigated in all surgical branches including urologic surgery (1). Fibrin glue is both available commercially and could be produced by blood banks autologously (1,2). Fibrin glue is used widely as a local haemostatic agent and tissue connecter which could be combined with the traditional suture techniques. Fibrin glue can be dissolved effectively and suggested that not causing major tissue fibrosis, inflammation and foreign body reaction. It could be absorbed within days (3,4). Fibrin glue has a stimulant effect on angiogenesis, local tissue growth and wound reparation (2). Adhesive power of fibrin glue depends on the fibrin concentration. The optimal adhesive effect could be reached in 3 to 5 minutes. Moreover, it has a positive effect on wound healing due to its fibronectin content. The wound healing effect is started by the fibroblasts migrating to the coagulum in the matrix forming surrounding tissue. The collagen synthesis made by the fibroblasts starts the connective tissue formation. The glue has to be absorbed during the wound healing process. The resorption depends on the amount of the applied fibrin glue, fibrinolytic activity and the phagocytosis of the macrophages. The tissue plasminogen activators convert plasminogen into plasmin and this converts the crosswise bound fibrin into the soluble fibrin products (2,3).

The production techniques of fibrin glue are still controversial due to the contamination risk of viral hepatitis, AIDS and the other serologically transmitted diseases. Because of these reasons, fibrin glue is synthesized from autologous blood and solitary donor blood (2). The safety and effectiveness of the fibrin glue in different surgical procedures is still highly controversial. In our study, we studied the safety and effectiveness of commercial fibrin glue for the repairment of artificial urinary bladder incisions.

Materials and methods

Thirty adult Wister-albino male rats (body weight 230-270 g) obtained from Firat University Medical Faculty Experimental Research Center (FÜTDAM) were randomly separated into three groups, each containing 10 rats following approvals by local committee. All animals were housed in a temperature- and light-controlled room with ad libitum access to water and rat chow except from 8 hours prior to 24 hours after the operation during the whole study. All surgical procedures were performed under xylasine (2.15 mg/kg) and ketamine (30 mg/kg) anaesthesia. Each animal was positioned supine and fixed, and then sterilely prepared and draped. Skin and other layers of the lower abdomen were passed with a midline vertical incision. The fascia was opened and the muscles were slipped off with blunt dissection. The bladder was exposed after opening the peritoneum. A 5 mm incision was made to the bladder of all subjects. Fibrin glue mixture (TISSEEL KIT 1.0 Österreichisches Institut Für Haemoderivate GES. M. B. H. Produced Immuno AG, Vienna, Austria) was prepared accordingly.

For the first group (suture only), the bladder incision was sutured continuously with 5/0 Dexon. Then the abdomen was closed separately through peritoneal and fascia sutures with 3/0 chromized catgut and the skin was closed with 3/0 silk suture. For the second group (fibrin glue only), the lips of the bladder incision was approximated by two transient sutures, and then the glue was applied. The sutures were removed 5 minutes after the application. Then the layers were closed. For the third group (suture plus fibrin glue), the lips of the incision was closed by 5/0 Dexon stay sutures after application of fibrin glue, and then the anatomical layers were closed. Amikasin sulphate (100 mg/2ml, 15mg/kg/day) (Bristol-Myers Squibb Company Co) was applied intramuscularly to all subjects for prophylaxis for two days. All the available animals were anesthetized with the same technique after four weeks of observation. Their abdomens were shaved and cleaned with batticon solution. A plastic 2G angiocath is placed into the urethra, contrast dye (10% urographin) in 0.9% NaCl solution is applied and cystography is performed (55 kv,12 mAs exposure, AP position). After this, laparotomies for cystectomy were performed for histological examination.

The animals were treated under the Guidelines for Animal Research at Firat University Faculty of Medicine, and in accordance with the Guide for the Care and Use of Laboratory Anaimals. The specimens were stained with Masson's trichrome technique for detection of fibrosis together with Haematoxylin-Eosin. The histological sections were examined using light microscopy (Olympus BX50) and graded on a scale of 0 to 3 (no, minimal, moderate and high) for the presence and degree of fibrosis, oedema, inflammation and bleeding.

Kolmogorov-Smirnov test was used for confirmation of normality. Than one-way ANOVA test was used for comparisons of groups and Tukey HSD multiple comparison test was used for defining which group makes the difference. The tests were conducted by using SPSS 11.0 software program.

Results

In the suture only group, one animal died on the third and another on the sixth day postoperatively and autopsies revealed urinoma. In the fibrin glue only group, two died on the 5th day, one died on the 8th day and one died on the 10th day postoperatively.. In the suture plus fibrin glue group, one animal died on the second and another one died on the 5th day postoperatively, again autopsy examinations revealed urinoma. There was no significant difference for deaths among the groups. Retrograde cystography examinations of all the living animals in each group revealed minor changes at the end of the one month. There was no extravasation of contrast material and small anatomic irregularities at the surgical repair site in the suture only group animals (Figure 1a). Extravasation of the contrast was not also observed in the fibrin glue only repaired animal, but the irregulari-



Figure 1. Retrograd cystographies obtained one month after closure of bladder incisions. a Lateral film showing minimal changes on the incision site in a suture only repairment group animal. b *A*-*P* film showing minimal changes on the incision site in a fibrin glue only repairment group animal.

ties at the surgical repair site were more prominent (Figure 1b). Anatomic irregularities were also minimal at the surgical site of the animals repaired with suture plus fibrin glue. Vesicoureteral reflux was not detected in any of the animals.

The results of histological examination were presented in Table 1. Histological examination revealed high grade fibrosis in one, moderate fibrosis in five and minimal fibrosis in two animal of the suture only group.

| Histologic Finding | Degree | Suture only (GI) | FG only (GII) | FG + Suture (GIII) | P (one-way ANOVA) | |
|--------------------|-------------|---------------------|------------------|-----------------------|----------------------|--|
| | no | 0 | 0 | 0 | | |
| Eibrogia | 1+ | 2 | 1 | 2 | 0.082 | |
| FIDIOSIS | 2+ | 5 | 0 | 5 | 0.082 | |
| | 3+ | 1 | 5 | 1 | | |
| | no | 0 | 5 | 3 | | |
| Osdama | 1+ | 3 | 1 | 4 | 0.001 | |
| Oedema | 2+ | 5 | 0 | 1 | 0.001 | |
| | 3+ | 0 | 0 | 0 | | |
| | no | 0 | 1 | 0 | | |
| Inflormation | 1+ | 1 | 5 | 3 | 0.001 | |
| Innamination | 2+ | 3 | 0 | 4 | 0.001 | |
| | 3+ | 4 | 0 | 1 | | |
| | no | 3 | 5 | 3 | | |
| Dlaading | 1+ | 5 | 1 | 4 | 0.176 | |
| Bleeding | Bleeding 2+ | | 0 | 1 | 0.170 | |
| | 3+ | 0 | 0 | 0 | | |

Table 1. Distribution of fibrosis, oedema, inflammation and bleeding on the healing site of the suture only(GI), fibrin glue only(GII) and fibrin glue plus suture group (GIII) animals by histologic examination

GI: Group I; GII: Group II; GIII: Group III; FG: Fibrin Glue; (no): no evidence; (1+): mild; (2+): moderate; (3+): high



Figure 2. Moderate fibrosis, obvious inflammation and mononuclear infiltration at the surgical site of an animal in the suture only repaired group. (a H&E and b Masson's Trichrom, X40)

Moderate fibrosis, obvious inflammation and mononuclear infiltration at the surgical site of an animal in the suture only repaired group are shown in Figure 2. There were high grade fibrosis in five, and minimal fibrosis in one animal of the fibrin glue only group. Prominent fibrosis and minimal inflammation at the surgical site of an animal in the fibrin glue only repaired group are shown in Figure 3. In the suture plus fibrin glue group, one animal had high grade, 5 had moderate and two had minimal fibrosis. Development of fibrosis was not differed significantly (P = 0.082) among the groups. Moderate fibrosis, minimal oedema, mononuclear infiltration and bleeding points around the lamina propria at the surgical site of an animal in the suture plus fibrin glue repaired group are shown in Figure 4.



Figure 3. Prominent fibrosis and minimal inflammation at the surgical site of an animal in the fibrin glue only repaired group. (a Hematoksilen Eozin and b Masson's Trichrom, X40)





Figure 4. Moderate fibrosis, minimal oedema, mononuclear infiltration and bleeding points around the lamina propria at the surgical site of an animal in the suture plus fibrin glue repaired group. (a Hematoksilen Eozin and b Masson's Trichrom, X40)

Three animals had minimal and five had moderate oedema in the suture only group. Five animals had no evidence of and one had minimal oedema in the fibrin glue only group. Three animals had no evidence, four had minimal and one had moderate oedema in the suture plus fibrin glue group. The oedema development among the groups was differed significantly (P = 0.001). Oedema was developed significantly more in suture only animals than fibrin glue only (P < 0.001) and than that of suture plus fibrin glue animals (P = 0.17). One animal had minimal, three had moderate, and four had high grade inflammation in the suture only group. One animal had no evidence of and five had minimal grade inflammation in the fibrin glue only group. Three animals had minimal, four had moderate and one had high grade inflammation in the suture plus fibrin glue group. There was significant difference among the groups for the development of inflammation (P = 0.001). Development of inflammation was significantly lower in fibrin glue only animals than that of suture only group (P = 0.001) and than that of suture plus fibrin glue group (P = 0.046).

Three animals had no evidence of bleeding and five had minimal amount of bleeding in the suture only group. Five animals had no evidence and one had minimal grade bleeding in the fibrin glue only group. Three animals had no evidence, four had minimal and one had moderate amount of bleeding in the suture plus fibrin glue group. There was no significant difference among the groups for bleeding (P = 0.176).

Discussion

Although, the death rates among our animal groups were not significantly different, the relative risk of death for the animals repaired only with fibrin glue was 2.22 times higher. Marcovich et al (4) have also used fibrin glue for closure of cystotomy in an experimental study and reported more deaths in the fibrin glue used animals, though their finding was also not reached significance. Our histological examination findings disclosed that fibrin glue only closure results in meaningfully less oedema and less inflammation at the repair site of bladder incision. Zilling at al (5) analyzed the strength of; fibrin glue, suture and staples used for small bowel anastomosis in pigs, and reported that the strength of fibrin glue anastomosis had only one fifth of the stapled and one third of the sutured ones. Anidjar et al (6) used fibrin glue for urethral anastomosis in an experimental study and reported that without combination of

fibrin glue with surgical suture may not be secure. Moreover, fibrin glue was suggested (5,6) as an excellent sealant and haemostatic agent, though not ideal for wound approximation unless it is small and has low tensile pressure. Our results confirms the suggestions made above that fibrin glue shouldn't be used solely for the closure of urinary bladder incisions, but it may be used in combination with surgical sutures in order to prevent urinoma formations which is the main reason for mortality at least in animals. Fibrin glue is clinically and experimentally used in almost all surgical branches including urology. Papledopoulas et al used fibrin glue for artificial vesicovaginal fistula repairment in a rabbit model and reported no recurrence in the fibrin glue plus suture combination group animals (7). In clinical studies, fibrin glue is successfully used for haemostatic control of hepatic and splenic incision and vascular anastomosis (8). In urology, the use of fibrin glue was reported for renal surgery (9,10), hipospadias repairment (11,12), pyeloplasty (13), ureteral anastomosis (13), urinary fistula (7) and vasovasostomy (14). Moreover, the use of fibrin glue as haemostatic in surgeries such as vesicouretheral reflux (1), circumcision of the haemophilic patients (15), suspension of the bladder neck (16) and prostatectomy (17) were also reported. Rossi et al (18) reported successful repairment of three patients with postoperative urinary fistulas by use of fibrin glue. Evans et al (19) reported successfully treatment (94.7%) of urinary tract injuries occurring in seven patients during general surgery and gynecological surgery, in five patients with complex urinary fistulas and in five patients during urologic surgery by direct injection of fibrin glue. Kram et al (9) used fibrin glue in the repairment of 14 patients with renal trauma and reported no renal loss, infection, late bleeding and urinary leakage in any of their patients.

Conclusion

The safety and effectiveness of fibrin glue for tissue approximation and repairment is still controversial. Our results may suggest that fibrin glue may be used together with suturing for better healing and safer closure of bladder incisions. Development of more potent tissue adhesives should be encouraged to expand reconstructive potential of surgical techniques.

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Impact of autologous plateletphoresis combined with intraoperative autologous blood salvage on oxygen transport in patients undergoing internal fixation of spine

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Abstract

Aim: To explore impact of autologous plateletphoresis (PLT) with intraoperative autologous blood salvage (ABS) on oxygenation in patients undergoing internal fixation of spine.

Methods: 200 patients were divided into four groups: Group I (preoperative acute PLT), Group II (Group I with intraoperative ABS), Group III (acute normovolemic hemodilution with ABS)and Group IV (intraoperative ABS). Indicators like oxygen delivery, mixed venous oxygen saturation, oxygene consumption, oxygen extraction ratio, gastric intramncosal PH were monitored.

Results: The postoperative 24 h drainage volume was less in Group I and II than those in Group III and IV (P < 0.05), while PLT indicators higher in Group I and II than in group III and IV at T_4 - T_5 (P < 0.05); The allogeneic transfusion rates in Groups were 0, 4%, 20%, and 68% respectively. There were no significant changes in monitoring indicators among four groups.

Conclusion: Preoperative PLT transfusion with intraoperative ABS won't affect tissue oxygenation and reduce intraoperative and postoperative need for allogeneic blood.

Key words: Platelets, apheresis, blood transfusion, autologous, oxygen.

Introduction

Autologous platelet (PLT) transfusion can reduce blood use in clinical settings(1), while few Chinese literatures have discussed the impact of preoperative autologous plateletphoresis combined with intraoperative autologous blood salvage on tissue oxygen metabolism. In this study, we tried to explore the impact of autologous plateletphoresis combined with intraoperative autologous blood salvage on oxygen transport in patients undergoing internal fixation of spine, with an attempt to validate its feasibility.

Methods

Patients

This study was approved by the Ethics Committee of Ningbo No.1 Hospital, and all patients participated have signed the informed consent. Totally 200 Patients undergoing internal fixation of spine [American Society of Anesthesiologists (ASA) grade I-II], aged 25-65 years, weighing 55-75 kg, were enrolled in this study. Other baseline indicators included: preoperative hemoglobin (Hb) > 13 g/dl, hematocrit (Hct) > 33%, normal platelet and coagulation functions, and without a history of anticoagulant usage within the past two weeks. No pulmonary, hepatic, renal and hematological complications were observed. Patients were randomly divided into four groups: group I (n=50), receiving preoperative acute autologous platelet-rich plasma (APRP) apheresis (direct acquisition) combined with intraoperative autologous blood salvage; group II (n=50), preoperative acute autologous plateletphoresis (acquisition of whole blood contained in bags following normovolemic hemodilution) combined with intraoperative autologous blood salvage; group III (n=50), acute normovolemic hemodilution combined with autologous blood salvage (plateletphoresis not conducted); and group IV (n=50), simple intraoperative autologous blood salvage.

Management

Establish the valid monitoring before induction

Patients received left radial artery cannulation under local anesthesia after entering the operating room for continuous blood pressure measurement and right internal jugular venipuncture for placement of the Swan-Ganz catheter (CCO/ $S\overline{v}$ O_2) to monitor continuous cardiac output (CCO) and mixed venous oxygen saturation ($S\overline{v} O_2$) using the continuous cardiac output monitor (CCO-Baxter Vigilance, Baxter Healthcare). The gastric tonus test tube was placed prior to induction of anesthesia to a certain depth; after suction of gastric juice was patent or the catheter position was confirmed to be correct by X-ray, a gastric tonus determinator (Tonocap, Datex-Ohmeda, Helsinki, Finland1 Type TC22001021 EN) was connected.

Anesthesia

Anesthesia was induced by intravenous injection of midazolam 0.05 mg/kg, propofol 1.5-2.0 mg/kg, fentanyl 3-5 μ /kg, and vecuronium 0.1 mg/kg, and maintained by continuous intravenous pump infusion of fentanyl 5 - 10 μ g • kg⁻¹·h⁻¹ and propofol 3-4 mg • kg⁻¹·h⁻¹ as well as inhalation of 0.5%-1.0% isoflurane; muscle relaxation was maintained by intermittent intravenous infusion of vecuronium.

Autologous platelet acquisition

Blood was collected via the right internal jugular vein after endotracheal intubation and 10 minutes of maintenance of stabilization.

Platelet-rich plasma apheresis (direct acquisition): Plateletphoresis (platelet apheresis) was conducted on the collected autologous whole blood in compliance with direct acquisition procedures using the Model Cell Saver®5 Autologous Blood Recovery System (Haemonetics Corporation, USA) and the corresponding disposable platelet-rich plasmapheresis consumables; 500-700 ml blood was needed for one extraction cycle, from which 30-50 ml platelets were extracted; in total about four cycles were performed. The speed of bleeding was controlled within 50-100 ml/min. Sodium Lactate Ringer's Injection and succinylated gelatin were used for fluid expansion via the peripheral vein at the same time, so as to maintain hemodynamic stability. The remaining blood components (plateletpoor plasma and red blood cell/RBC concentrates) were transfused back into the patient's body at the end of each cycle, or platelet-poor plasma and RBC concentrates were transfused during the operation based on the change of Hb.

Plateletphoresis (indirect acquisition): Plateletphoresis was conducted on the collected autologous whole blood (whole blood collected following normovolemic hemodilution) in compliance with whole blood bag acquisition procedures using the Model Cell Saver®5 Autologous Blood Recovery System (Haemonetics Corporation, USA) and the corresponding disposable platelet-rich plasmapheresis consumables. Platelet concentrates were stored using a platelet vibration preserving machine at 22 °C within a constant temperature oscillation freezer. The units of platelets to collected was predicted, namely, 25% greater than the estimated total units of platelets (total units of platelets = pre-acquisition platelet count \times estimated blood volume). The collected platelet concentrate samples were kept to calculate the actual number of collected platelets. Autologous platelet transfusion: APRP collected was transfused by stages after the operation (no obvious hemorrhage was observed at the surgical field).

Intraoperative autologous blood salvage: In groups I-IV, blood shedding from the surgical field was recovered using the Model Cell Saver®5 Autologous Blood Recovery System (Haemonetics Corporation, USA) during the operation. When the level of Hb was less than 80 g/L and if collection of washed autologous RBC concentrates was still under way, allogeneic RBC concentrates were transfused in advance; the washed RBC concentrates were transfused prior to APRP transfusion.

Monitoring indicators

The intra-operative heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP) and electrocardiogram (ECG) were continuously monitored intra-operatively. CCO and $S\overline{v}$ O_2 were monitored using the CCO monitor (CCO-Baxter Vigilance, Baxter Healthcare).

Tissue oxygenation indicators: Mixed venous blood and arterial blood were collected immediately prior to plateletphoresis (T_1), immediately after plateletphoresis (T_2), immediately prior to autologous blood transfusion (T_3), 10 min after APRP transfusion (T_4), and 1 h after the operation (T_5) for blood gas analysis in groups I and II. The cardiac index (CI), oxygen delivery (DO₂), oxygen consumption (VO₂), and oxygen extraction ratio (ERO₂) were calculated. The concentration of arterial blood lactate was monitored.

Gastric intramucosal pH (i-pH) measurement by gastric tonometry: The determinator allowed automatic inflation of the silicon capsule with 5 ml air, so that CO₂ could realize free balance among the gastric mucosa, gastral cavity and air-containing capsule; air was extracted from the air-containing capsule every 10 min to determine gastric intramucosal carbon dioxide (CO₂) tension (PiCO₂) using the infrared sensor technology. After entering arterial pH (pHa) and arterial CO₂ tension (PaCO₂) at corresponding time points, we could get i-pH at these time points by the instrument.

In group III and IV, the above mentioned indicators were determined 10 min after stabilization of anesthesia induction, 50 min after anesthesia, immediately before autologous blood transfusion, immediately after the operation, and 1 h after the operation.

The intraoperative salvaged volume of autologous blood, amount of bleeding (blood volume in gauze was determined according to the drainage volume within the suction bottle and the weighing method), volume of transfusion, and drainage volume and blood transfusion 24 h after the operation were recorded.

Intraoperative autologous blood salvage: In group I-IV, blood shedding from the surgical field was recovered using the Model Cell Saver®5 Autologous Blood Recovery System (Haemonetics Corporation, USA) during the operation. When the level of Hb was less than 80 g/L and if collection of washed autologous RBC concentrates was still under way, allogeneic RBC concentrates were

transfused in advance; the washed RBC concentrates were transfused prior to APRP transfusion.

Statistical analysis

All data were statistically analyzed using SPSS 13.0 statistical software. Measurement data were expressed as $\overline{x} \pm s$. Intra-group differences were compared by one-way analysis of variance (ANO-VA), and inter-group differences were compared using the two independent samples *t* test. Enumeration data were analyzed with the $\chi 2$ test. A *P*-value of less than 0.05 was considered statistically significant.

Results

Differences in the sex ratio, age, body weight and preoperative Hb, Hct and platelet count (Plt) among the four groups were not statistically significant. In addition, the mean amount of blood loss and autologous blood transfusion also showed no significant difference. The postoperative 24 h drainage volume was significantly less in group I and II than those in group III and IV (P < 0.05). The allogeneic transfusion rates were 0, 4%, 20%, and 68% in group I, II, III, and IV (Table 1).

Tissue oxygenation indicators In the four groups, DO₂ was significantly lower at T₃ than T₁ (P < 0.05) and ERO₂ was significantly higher at T₃ than T₁ (both P < 0.05). There were no significant changes in S⁻v O₂ and VO₂ at different time points (P > 0.05) and among these four groups (Table 2).

The arterial blood lactate levels in the four groups were significantly higher at T_3 than T_1 (P < 0.05) and there were no obvious changes at other time points. Inter-group comparison showed no significant differences (P > 0.05) (Table 3). Values of i-pH in the four groups were lower at T_3 than

Table 1. Comparison of the perioperative amount of blood loss, autologous blood transfusion and allogeneic blood transfusion (ml) among four groups (n = 50, $\overline{x} \pm s$)

| Group | Intraoperative amount of blood loss | Amount of autologous blood transfusion | Intraoperative amount of blood transfusion | Postoperative 24 h amount of bleeding | Postoperative amount of allogeneic blood transfusion | |
|-----------|---|--|--|---|--|--|
| Group I | 895±237 | 667±118 | 3121±193 | 115±31* | 0† | |
| Group II | 905±200 | 688±131 | 2902±203 | 230±33 | 105±82 | |
| Group III | 918±189 | 665±109 | 3180±211 | 293±42 | 201±71 | |
| Group IV | 934±169 | 694±101 | 2890±227 | 365±43 | 288±68 | |

Compared with group II, III and IV, $*P < 0.05 \ \dagger P < 0.01$.

| Table 2. | Changes | in oxygen | metabolism | and l | blood | lactate | of th | he four | groups | at e | different | time | points |
|-----------|----------|-----------|------------|-------|-------|---------|-------|---------|--------|------|-----------|------|--------|
| (n=50, x) | $\pm s)$ | | | | | | | | | | | | |

| Indicators | Group | T ₁ | T ₂ | T ₃ | T ₄ | T ₅ |
|---|-----------|----------------|----------------|----------------|-----------------|----------------|
| | Group I | 3.4±0.6 | 3.7±0.9 | 3.8±0.4 | 3.7±0.5 | 3.8±0.6 |
| $CI(I,min^{-1},m^{-2})$ | Group II | 3.5±0.7 | 3.6±0.9 | 3.7±0.6 | 3.8±0.5 | 3.7±0.9 |
| | Group III | 3.6±0.5 | 3.4±0.8 | 3.9±0.8 | 3.8±0.9 | 3.9±0.4 |
| | Group IV | 3.7±0.8 | 3.6±0.6 | 3.8±0.6 | 3.7±0.6 | 3.8±0.8 |
| | Group I | 588±101 | 599±103 | 476±71 ‡ | 584±71 | 581±120 |
| $DO(m1 min^{-1} m^{-2})$ | Group II | 559±101 | 584±111 | 460±69 ‡ | 579±71 | 579±108 |
| $DO_2(111,1111,1111,111,1)$ | Group III | 601±100 | 580±112 | 470±71 ‡ | 591±69 | 603±110 |
| | Group IV | 568±101 | 576±110 | 484±70 ‡ | 601±72 | 597±109 |
| | Group I | 125±26 | 124±20 | 118±18 | 125±18 | 126±17 |
| VO(ml.min-1.m-2) | Group II | 121±25 | 126±21 | 121±18 | 127±17 | 125±18 |
| $VO_2(111111111111111111111111111111111111$ | Group III | 120±23 | 121±19 | 123±19 | 123±18 | 129±19 |
| | Group IV | 123±19 | 119±21 | 124±17 | 126±19 | 120±17 |
| | Group I | 21.3±2.1 | 20.7±1.8 | 24.9±2.5 ‡ | 21.4±1.9 | 21.8±1.7 |
| $EPO(\theta/)$ | Group II | 21.7±2.6 | 21.6±2.0 | 26.1±1.9 ‡ | 21.8±0.5 | 21.6±2.1 |
| $EKO_2(70)$ | Group III | 19.9±2.0 | 20.9±1.9 | 26.2±2.2 ‡ | 20.8±1.6 | 21.5±1.8 |
| | Group IV | 21.7±2.3 | 20.7±1.7 | 25.7±1.8 ‡ | 20.9±2.3 | 20.1±2.4 |
| | Group I | 75.3±3.9 | 76.3±3.9 | 77.1±2.4 | 76.3±3.6 | 81.2±2.2 |
| S^{-} U $(0/)$ | Group II | 76.7±3.4 | 75.1±3.2 | 77.8±3.3 | 75.1±2.9 | 79.1±3.1 |
| $5 VO_2(70)$ | Group III | 73.5±4.2 | 74.6±4.6 | 76.2±4.1 | 74.2±3.1 | 76.3±3.3 |
| | Group IV | 74.2±3.6 | 75.2±5.4 | 77.1±3.5 | 73.9±2.6 | 74.1±2.8 |
| | Group I | 1.18±0.36 | 1.16±0.24 | 1.57±0.31 ‡ | 1.27±0.56 | 1.28±0.39 |
| $I \wedge (mmol/I)$ | Group II | 1.21±0.42 | 1.11±0.37 | 1.71±0.39 ‡ | 1.38 ± 0.31 | 1.39±0.42 |
| | Group III | 1.16±0.34 | 1.21±0.28 | 1.69±0.28 ‡ | 1.40 ± 0.28 | 1.40±0.31 |
| | Group IV | 1.23±0.33 | 1.28±0.31 | 1.74±0.31 ‡ | 1.29±0.43 | 1.43±0.41 |

Compared with T_p , $\ddagger P < 0.05$

Table 3. Comparison of *i*-pH, PiCO₂ and platelet count (Plt) of patients in the four groups at different time points (n=50, $x\pm s$)

| Indicators | Group | T. | T. | T. | T. | T. |
|-----------------------|-----------|-------------|-------------|---------------|-------------|-------------|
| | a a | -1 | -2 | -3 | -4 | -5 |
| | Group I | 7.443±0.037 | 7.446±0.062 | 7.362±0.037 ‡ | 7.418±0.049 | 7.457±0.067 |
| inU | Group II | 7.406±0.087 | 7.423±0.028 | 7.336±0.057 ‡ | 7.407±0.085 | 7.413±0.024 |
| і-рп | Group III | 7.392±0.056 | 7.373±0.076 | 7.313±0.087 ‡ | 7.381±0.059 | 7.385±0.104 |
| | Group IV | 7.433±0.091 | 7.386±0.033 | 7.319±0.055 ‡ | 7.403±0.077 | 7.405±0.029 |
| PiCO ₂ | Group I | 41.3±5.3 | 42.2±2.7 | 48.6±6.2 ‡ | 43.4±2.4 | 41.2±6.6 |
| (mmHg) | Group II | 38.8±3.1 | 40.7±7.8 | 46.6±2.3 ‡ | 41.3±7.7 | 39.4±5.3 |
| | Group III | 38.3±5.2 | 38.8±4.6 | 46.9±7.5 ‡ | 39.1±6.3 | 42.3±2.4 |
| | Group IV | 41.6±6.3 | 40.8±1.3 | 47.5±4.2 ‡ | 40.7±2.4 | 42.9±3.1 |
| | Group I | 180±33 | 179±32 | 136±22 | 190±27* | 210±13* |
| Plt | Group II | 178±18 | 174±12 | 128±17 | 161±12 | 158±19 |
| (×10 ⁹ /L) | Group III | 174±19 | 169±24 | 119±21 | 120±21 | 125±11 |
| Ì Ì | Group IV | 181±12 | 171±21 | 109±18 | 110±12 | 108±27 |

Compared with T_{p} $\ddagger P < 0.05$; compared with group II, III and IV, *P < 0.05.

 T_1 (P < 0.05) and PiCO₂ was significantly higher at T_3 than T_1 (P < 0.05), but there were no obvious changes at other time points. Inter-group comparison showed no significant differences (P > 0.05) (Table 3).

Plt indicators Compared with groups III and IV, Plt indicators significantly increased in groups I and II at T_4 - T_5 (P < 0.05); compared with group II, group I had significantly increased Plt indicators (P < 0.05) (Table 3).

The preservation and usage of autologous blood has played an increasingly important role in controlling allogeneic blood transfusion-associated problems such as infectious diseases and immune suppression. Ekback et al(2) have found that favorable therapeutic effects can be obtained through extraction of platelet-rich plasma prior to total hip replacement; by doing so, postoperative platelets are remarkably increased and the amount of bleeding is dramatically reduced, both of which are favorable for early wound healing. However, the volume of simple intraoperative autologous blood salvage is limited. It has been reported in literatures that Hb, Hct, coagulation function indicators, and platelet count can be significantly reduced after the operation(3). Serrick et al(4) have reported that the rate of platelet clearance of the Model Cell Saver®5 Autologous Blood Recovery System (Haemonetics Corporation, USA) was 86%, since the process of centrifugal washing can induce platelet destruction and a large number of coagulation factors are washed out and discarded. When a certain amount of salvaged autologous blood is transfused, since RBC concentrates have no haemostatic ability, hemorrhagic coagulation can be generated without reducing the postoperative amount of bleeding; when APRP is transfused following the transfusion of washed RBC concentrates, the number of in vivo platelets can be rapidly elevated and the fresh coagulation factors can be supplemented, reducing the postoperative amount of bleeding and thereby avoiding or reducing allogeneic blood transfusion. It was also observed in this study that the number of platelets in patients receiving APRP transfusion in group I was significantly higher than those in the other three groups, because after four cycles of direct plateletphoresis in group I, the acquisition efficiency was high, platelet count was significantly higher than that in the other three groups, postoperative amount of bleeding was significantly reduced, and no postoperative allogeneic blood was transfused. In group II, plateletphoresis on the whole blood collected by acute normovolemic hemodilution could elevate the number of platelets to a certain extent, but due to the limitation of the amount of whole blood collected, the platelet acquisition efficiency was lower than that in group I and the rate of allogeneic blood transfusion was 4%. In group III, autologous whole blood transfusion was conducted. The fresh coagulation factors could reduce postoperative bleeding, but the number of platelets was significantly lower than that in group I and the rate of allogeneic blood transfusion was 20%. All these findings suggest that transfusion of platelets obtained through direct preoperative plateletphoresis in combination with autologous blood salvage can reduce intraoperative and postoperative need for allogeneic blood in a more effective way.

Balance between the body's oxygen delivery and consumption can be clinically evaluated by monitoring S⁻v O₂, DO₂, VO₂ and ERO₂ and by determining the level of lactate. DO, is affected by CI, Hb, and SaO₂ and the threshold value of DO₂ is 330 ml·min⁻¹·m⁻². When DO₂ is less than 330 ml·min⁻ ¹·m⁻², reduction in DO₂ can cause the obvious reduction of VO₂, which can not maintain normal tissue oxygenation and thus lead to physiological oxygen delivery dependency(5). The results of this study have indicated that APRP acquisition as well as DO2, VO2 and ERO2 during plateletphoresis were basically consistent with those prior to the operation, because it is possibly that timely transfusion of RBC concentrates after each cycle can maintain Hb at relatively high levels and increase the oxygen-carrying capacity of RBCs, so as to maintain DO2 at high levels without posing any obstacle to tissue oxygenation. DO2 was decreased and ERO2 was increased before autologous blood transfusion, since Hb was reduced followed by a compensatory increase in ERO2 during hemodilution, but DO2 was still higher than the threshold value and oxygen delivery of the body could still meet the need of tissue. The level of arterial blood lactate is a sensitive and accurate indicator reflecting the balance between tissue organ oxygen delivery and consumption. In this study, the level of blood lactate was not insignificantly increased after APRP apheresistransfusion. Although it was higher at T₃ than T₁, the level was still within the upper limit of normal, indicating that this technology has no great impact on tissue hemoperfusion and will not induce microcirculatory disturbances. Continuous monitoring of S^v O₂ can dynamically reflect the balanced status of tissue oxygen delivery and consumption, which is mainly affected by CO, Hb, SaO_2 and, VO_2 during the perioperative period(6). In this study, continuous monitoring of S⁻vO₂ further suggested that DO₂ and VO₂ showed no obvious changes after autologous platelet acquisition, indicating that normal oxygen-carrying capacity can be maintained during the four cycles of acquisition.

Gastric intramuscular pH (i-pH) is a sensitive early indicator that reflects local tissue anoxemia of the gastrointestinal tract and systemic hypovolemia(7). Hypovolemia may reduce gastrointestinal mucosal perfusion of the body to ensure perfusion of vital organs. Thus, monitoring of gastrointestinal mucosal perfusion can contribute to early detection of hypovolemia. In this study, there were no obvious changes in i-pH and PiCO₂ after APRP apheresis-transfusion. Although i-pH was lower at T_1 than T_1 and PiCO₂ was higher at T_2 than T_1 , they were both within the upper and lower limits of normal, suggesting that this technology has no great impact on local tissue hemoperfusion of the gastrointestinal tract. By comparing preoperative autologous blood preservation, acute normovolemic hemodilution, surgical field bleeding or drainage tube blood salvage, autologous plateletphoresis extraction-transfusion, and other blood conservation measures, Hardy et al(8) have found that autologous plateletphoresis extraction-transfusion technology, with minimal impact on hemodynamics of the body and blood oxygen-carrying capacity, can effectively promote the recovery of coagulation functions and reduce postoperative bleeding. Their findings were also confirmed in this study.

To conclude, preoperative autologous platelet apheresis-transfusion combined with intraoperative autologous blood salvage will not adversely affect tissue oxygenation. The combined application can effectively reduce blood usage in clinical setting, and is a relatively safe and feasible method for blood conservation.

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Association between thyroid hormones and prognostic factors in thyroid carcinomas

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Abstract

Objective: In this study, we aimed to identify the prevalence and association of thyroid-stimulating hormone (TSH), free T3 (fT3), free T4 (fT4) levels among patients with thyroid papillary carcinoma (TPC), and papillary microcarcinoma (PMC) with the prognostic determinants like tumor size, multifocality intra- and extrathyroidal spread.

Patients and Methods: For the patients having thyroid malignancies, type and size of tumor, presence or lack of intra-thyroidal spread, association with multifocality, and presence of metastasis to lymph nodes were also recorded. The results of TSH, fT3 and fT4 of patients, at the time of biopsy performed, were recorded.

Results: There was 145 patients diagnosed as TPC and PMC through 1685 patients. Serum fT3, fT4, TSH levels did not differ between TPC and PMC patients. Serum fT3, fT4, TSH also did not change according to tumor size and intra-thyroidal spread in TPC patients. However fT3 levels were higher in patients with TPC with extra-thyroidal invasion.

Conclusion: Serum fT3, fT4, TSH levels are not associated with TPC and PMC. Indeed these parameters do not change according to the prognostic determinants like tumor size, multifocality intra- and extrathyroidal spread.

Key words: Papillary thyroid carcinoma, papillary microcarcinoma, thyroid stimulating hormone.

Introduction

The malignant tumors of thyroid represent a proportion of 1% among all cancer types. It is one of the most common endocrine malignancies (1-4).

Its incidence is increasing more rapidly than other tumors. The worldwide incidence of palpable nodule is 5 % among women and 1 % among men, however, with the extensive use of high-resolution ultrasound, this rate rises up to 19-67%, being more common among women. The clinical diagnosis of thyroid nodules is of importance since 5-15% of them are malignant (5,6). In the United States of America, the thyroid cancer accounts for 0.17 % of cancer-related deaths in women and 0.26 % of cancer-related deaths in men (1). The mortality due to thyroid cancer is increasing. This is because early diagnosis and surgical treatment and/or effective treatment with I^{131} are provided (7, 8).

The majority of thyroid cancers (95 %) originate from the follicular cells. The differentiated thyroid cancers (papillary and follicular carcinomas) arise from the follicular cells and synthesize thyroglobulin. The thyroid carcinomas can be classified as well-differentiated carcinomas (90 %), medullary carcinoma (7 %), anaplastic carcinoma (3 %) and others. The well-differentiated carcinoma types involve papillary carcinoma (80 %), follicular carcinoma (10 %), papillary microcarcinoma and Hurthle cell carcinoma. The thyroid papillary carcinomas sized less than one centimeter are defined as papillary microcarcinoma (occult thyroid carcinoma) (1).

Enlarged irregular thyroid gland also occurs as a consequence of constant and intensive thyroidstimulating hormone (TSH) stimulation in case the functions of the thyroid gland are insufficient. Iodine deficiency, thyroid gland being incapable of producing hormone, goitrogens taken in diet or drugs rise the risk of thyroid cancer leading to an increase in TSH level (8). Hormone production in the thyroid gland, iodine uptake, and enlargement of thyroid gland depend on the effects of TSH on thyroid gland. The pituitary gland responds to any change in circulating levels of thyroid hormone, reducing or increasing the release of TSH. The TSH receptors are located on the follicular cell membranes (10).

TSH rises particularly in thyroid carcinomas accompanied by thyroiditis. Low TSH levels are observed in hyper functioning (toxic) nodules. Serum TSH level is non-specific for the diagnosis of thyroid cancer since it increases in many thyroid disorders. TSH stimulates growth or development in thyroid malignancies and it is known that high serum TSH level is associated with the incidence of thyroid cancer and advanced tumor stage. However, it is not known exactly the role of TSH in high risk properties in well-differentiated thyroid cancer. Thyroid hormone suppression therapy is associated with declined rates of recurrence and prolonged survival in differentiated thyroid cancer patients (9,10,11).

In this study, we aimed to identify the prevalence of thyroid papillary carcinoma (TPC) and papillary microcarcinoma (PMC) in Kayseri, a Middle Anatolia city in Turkey, and to assess the association of serum thyroid-stimulating hormone (TSH), fT3 and fT4 values between preoperative histopathological findings.

Materials and Method

For this study, the data belonging to total thyroidectomy cases evaluated in the Department of Pathology at Kayseri Training and Research Hospital were reviewed retrospectively. The diagnosis were based on the pathological evaluation of surgical specimen or fine needle aspiration biopsy. The study included totally 145 patients, who had been diagnosed as TPC (n=46) or papillary microcarcinoma (n=99), through 1685 patients who had total thyroidectomy in the period of October 2007 to December 2012. Histopathological diagnosis of thyroid biopsy specimens, pre-treatment TSH, fT3, fT4 values belonging to these patients were evaluated by extracting these data from the computerized database and medical files. The serum TSH, fT3 and fT4 levels were measured by enzyme immunoassay method on hormone autoanalyzer (Immulite 2000, DPC Diagnostics, Los Angeles, CA, USA). The reference ranges were accepted as 1.5-5.0 pg/ml for fT3, 0.8-1.7 ng/dl for fT4, and 0.35-5.5mlU/L for TSH. For the patients having malignancies, type and size of tumor, presence or lack of intra-thyroidal spread, association with multifocality, and presence of metastasis to lymph nodes were also recorded.

Statistical analysis

For statistical analyzes of data, Statistical Package for Social Sciences (SPSS) for Windows ver. 15 software was used. Shapiro-Wilk test was done to determine normal distribution. Chi-square analysis, two-way independent sample-*t* test and Mann-Whitney U test were used to compare the data. The values were presented as *n* (%), mean \pm standard deviation or median (25.-75. percentile). A statistical analysis level of p<0.05 was set to be significant.

Results

There was 145 patients diagnosed as TPC (n=46) and PMC (n=99) through 1685 patients. Serum fT3, fT4, TSH levels did not differ between TPC and PMC patients. The mean age was 49.7±11.8 years for TPC and 49.7±10.8 years for PMC. Among PMC patients, there were 8 men (8.1 %), and 91 women (91.9 %) of TPC patients, 8 (17.4 %) were men and 38 (82.6 %) were women (Table 1). The FNAB was performed to 33 (33.3 %) of 99 patients diagnosed with PMC. The diagnosis of these patients were cellular follicular lesion (n=10, 30.3 %), nodular colloid goiter (n=11, 33.3 %), suspected TPC (n=4, 12.1%), TPC (n=2, 6.1%), hemorrhagic cyst (n=2, 6.1 %), non-diagnostic (n=4, 12.1 %). Of 46 patients diagnosed as TPC, 27 patients were performed preoperative FNAB. The diagnoses for these patients are listed in Table 2. Surgical resection was recommended to 20 (60.6 %) of 33 patients with PMC and 12 (44.4 %) of 46 patients with TPC because of the diagnosis of suspected malignancy depending on FNAB examinations.

The mean TSH levels were found to be 0.92 mlU/L (0.56-1.52) for PMC and 1.32 mlU/L (0.65-2.23) for TPC (p<0.05). Among patients with TPC, the tumor size was less than 3 cm in 22 patients and larger than 3cm in 24 patients. Mean age was 51.8 ± 13.9 years among patients having a tumor size larger than 3cm and 49.2 ± 10 years among patients with tumors sized less than 3cm (p >0.05, Table 3).

| Variables | Papiller Microcarcinoma (<i>n</i> =99) | Papiller Carcinoma (<i>n</i> =59) | р | OR |
|------------------------|--|---------------------------------------|-------|-----------------|
| Age | 49.74±10.81 | 49.71±11.77 | 0.989 | 1.00(0.97-1.03) |
| Gender (male / female) | 8(8.1)/91(91.9) | 8(13.6)/51(86.4) | 0.270 | 1.78(0.63-5.04) |
| TSH (mlU/L) | 0.92(0.56-1.52) | 1.32(0.65-2.23) | 0.051 | 1.02(0.96-1.08) |
| fT3 (ng/dL) | 3.46(3.10-4.00) | 3.25(2.77-3.62) | 0.041 | 0.73(0.46-1.16) |
| fT4 (mlU/L) | 1.12(0.99-1.27) | 1.06(0.96-1.31) | 0.875 | 0.88(0.29-2.67) |

Table 1. Characteristics of PMC and TPC patients

Values are expressed as mean±*SD or median (25th-75th percentiles). OR: Odds Ratio*

Table 2. Preoperative FNAB results (n) of patients with PMC and TPC patients

| FNAB | Papillary Microcarcinoma (<i>n</i> =66) | Papillary Carcinoma (<i>n</i> =19) | |
|-------------------------------|---|--|--|
| Cellular follicular lesion | 10 | 3 | |
| Suspected papillary carcinoma | 4 | 7 | |
| Papillary carcinoma | 2 | 2 | |
| Non-diagnostic | 4 | 12 | |
| Nodular colloid goiter | 11 | 3 | |
| Hemorrhagic cyst | 2 | 0 | |

| Table 3. | Characteristics | of TPC | patients | according to | o tumor | size |
|----------|-----------------|--------|----------|--------------|---------|------|
|----------|-----------------|--------|----------|--------------|---------|------|

| Variables | Tumor size (<3cm) (<i>n</i> =22) | Tumor size (≥3cm) (<i>n</i> =24) | р |
|------------------------|--------------------------------------|--------------------------------------|-------|
| Age | 51.86±13.90 | 49.21±10 | 0.463 |
| Gender (male / female) | 3(13.6)/19(86.4) | 2(8.3)/22(91.7) | 0.659 |
| TSH (mlU/L) | 1.19(0.46-2.21) | 1.51(0.67-2.17) | 0.613 |
| fT3 (ng/dL) | 3.28(2.86-3.62) | 3.30(2.77-3.77) | 0.919 |
| fT4 (mlU/L) | 1.06(0.97-1.41) | 1.11(1.00-1.35) | 0.733 |

Values are expressed as mean \pm SD or median (25th-75th percentiles).

Table 4. Characteristics of TPC patients according to extra-thyroidal invasion

| Variables | Extra-thyroidal invasion (none) (<i>n</i> =35) | Extra-thyroidal invasion (present) (<i>n</i> =11) | р |
|------------------------|--|---|-------|
| Age | 50.54±11.96 | 50.27±13.13 | 0.949 |
| Gender (male / female) | 3(8.6)/31(91.4) | 2(18.2)/9(81.8) | 0.580 |
| TSH (mlU/L) | 1.30(0.46-2.21) | 1.33(1.01-2.12) | 0.741 |
| fT3 (ng/dL) | 3.21(2.75-3.58) | 3.62(3.36-4.69) | 0.002 |
| fT4 (mlU/L) | 1.09(1.00-1.35) | 1.03(0.80-1.41) | 0.244 |

Values are expressed as n(%), mean \pm SD or median (25^{th} - 75^{th} percentiles).

Table 5. Characteristics of TPC patients according to intra-thyroidal spread

| Variables | Intrathyroidal spread (absent) (<i>n</i> =38) | Intrathyroidal spread (present) (n=8) | р |
|------------------------|---|--|-------|
| Age | 50.50±12.94 | 51.50±10.33 | 0.979 |
| Gender (male / female) | 5(13.2)/33(86.8) | 0(0.0)/8(100.0) | 0.569 |
| TSH (mlU/L) | 1.45(0.75-2.21) | 1.06(0.55-1.71) | 0.400 |
| fT3 (ng/dL) | 3.30(2.86-3.73) | 3.29(2.77-3.59) | 0.782 |
| fT4 (mlU/L) | 1.11(1.00-1.41) | 1.06(0.86-1.12) | 0.201 |

Values are expressed as mean \pm *SD or median (25th-75th percentiles).*

Serum fT3 levels of patients having tumors invading adjacent tissues were significantly higher than the patients having tumors not invased (p<0.05, Table 4). Of 38 patients with TPC without intra-thyroidal spread, 5 (13.2 %) were men and 33 (86.8 %) were women. All 8 patients with intra-thyroidal spread were women (Table 5).

We could not find any significant correlation between preoperative TSH levels and tumor size, multifocality, co-existence of intra-thyroidal invasion in patients with TPC .

Discussion

In developed countries, estimated annual incidence of thyroid cancers is approximately 12 % per 122.000 cases among all cancers (3). Thyroid carcinomas account for 94.5 % of all new endocrine cancer cases and 65.9 % of deaths due to endocrine cancers; it has a slow course and long survival. The incidence of thyroid cancer rose from 3.6 per 100.000 to 8.7 per 100.000 in 2002. This rate rises by 6.2 % every year. This is because even very small cancers can be recognized. The mortality rate has remained stable at 0.5 death per 100.000 (9).

In this study we also assessed FNAB results which was performed in diagnostic work-up before surgical procedure, has already been recommended by guidelines and performed in everyday clinical practice in developed countries. Surgery was recommended because of the diagnosis of suspected malignancy depending on FNAB to 20 (60.6%) of 33 patients with PMC and 12 (44.4%) of 46 patients with TPC.

Thyroid cancers often occur in young and middle-aged adults, is rarely seen in children. The mean age when the disease develops is between 20 and 50 years of age for papillary carcinomas, 50 years of age for follicular carcinomas and 60 years of age for poorly differentiated or undifferentiated cancers (1, 2, 3). In our study, the median age was found to be 49.7±11.8 years for TPC and 49.7±10.8 years for PMC. The majority of studies have shown that thyroid cancers occur 2-4 times more frequently in women than men. This tendency is associated with sex hormones and less likely with the age of menarche (14,15). In this study, 91.9 % of PMC cases and 82.6 % of TPC patients were women.

Given the association of thyroid cancers with the functions of the gland, in the past, the presence of hyperthyroidism was accepted as a risk for thyroid cancer (3). The incidence of cancer among individuals with hyperthyroidism has been researched in many trials and was reported to be in a range from 0.2 to 21 % in the literature (10,11). In the studies researching the development and genetics of cancer among patients with hyperthyroidism, TSH was recognized as a significant factor (8). In situations where the functions of the thyroid are insufficient, an irregular enlargement of the thyroid gland occurs as a consequence of constant and intense TSH stimulation. Iodine deficiency, incapability of the thyroid gland to produce hormone, goitrogens ingested in diet or drugs increase the risk of thyroid cancer leading to a rise in TSH levels.

Hancock et al. claimed that prolonged TSH stimulation of the thyroid by goitrogens caused hyperplasia of the gland and eventually neoplasia. However, the evidences regarding the development of thyroid cancer due to excessive release of TSH are uncertain (10-16). The presence of TSH receptors in well-differentiated thyroid cancers endorses this opinion (17). Jonklaas J et al. verified that high TSH concentrations within normal limits were associated with subsequent diagnosis of thyroid cancer in individuals with thyroid abnormalities (18). This supports the hypothesis that TSH induces the growth of tumors in early or preclinical stage or the development of thyroid malignancy (12-14,18).

Kim SS et al. evaluated 554 patients diagnosed with differentiated thyroid cancer in a three-year period. Preoperative TSH level was found to be significantly higher in patients with extra-thyroidal spread (p=0.002) and with metastases to lymph nodes (p=0.007). As serum TSH concentration rises, the frequency of extra-thyroidal invasion also increases (p=0.009). It was found that the patients with a TSH level \geq 2.5 mIU / L had higher prevalence of extra-thyroidal invasion (p = 0.006) and lymph node metastases (p=0.024) (19).

In our study, the mean TSH concentrations were found to be 0.92 mlU/L (0.56-1.52) in PMC and 1.32 mlU/L (0.65-2.23) in TPC patients. However, this difference was not statistically significant. In addition, in patients with TPC, there was no significant correlation between preoperative TSH concentrations and tumor size, multifocality, and intrathyroidal invasion.

In a study, Haymart et al. investigated the association of TSH values and age with thyroid carcinoma. The authors found preoperative TSH concentrations to be significantly higher regardless of median age and similar to our results, they did not observe any correlation between TSH levels and age, tumor size, and metastases. The researchers found a significant correlation between poor prognosis and high TSH concentration, extra-thyroidal invasion (p=0.002) (20). In a study performed by Li et al., serum TSH levels, gender, age, type of tumor and number of tumors identified by ultrasound examination were evaluated retrospectively and their relationships with the incidence of thyroid cancer were investigated. Elevated serum TSH level was an independent risk factor for thyroid cancer (21).

With widespread use of imaging techniques, the incidence of PMC has been increased. Although most of the PMCs have excellent long-term prognosis, they may require aggressive treatment because of their tendency to metastasis. In the literature, there are few studies demonstrating the correlation between TSH and thyroid microcarcinoma (22). In a comprehensive study recently performed by Shi et al., higher serum TSH levels were found to be associated with differentiated thyroid carcinomas, but, not with PMCs (22).

Jonklass et al. found lower fT3 levels in thyroid carcinoma cases (18). However, that study was performed with very small number of patients (n=17). In contrast to Jonklass et al., with the present study, we demonstrated that fT3 levels were higher in TPC patients with adjacent tissue invasion (p=0.002). fT3 levels may rise in early period of subclinical hyperthyroidism. In that case, fT4 is usually normal, while TSH can be suppressed or normal (22). In subclinical hyperthyroidism, fT3 is often the first parameter being increased (23). Elevated fT3 level in our patients having adjacent tissue invasion might be a predictor anticipating hyperthyroidism to be developed in advanced stages.

In conclusion, serum fT3, fT4, TSH levels are not associated with TPC and PMC. Although these parameters do not change according to the prognostic determinants like tumor size, multifocality intraand extrathyroidal spread, fT3 levels were higher in TPC patients with adjacent tissue invasion.

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Expression of SULF1 expression in invasive breast ductal carcinomas and its clinical significance

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Abstract

Aim: The aim of the study was to investigate the expression of sulfatase 1 (SULF1) in invasive breast ductal carcinomas, furthermore their clinicopathological and prognostic values were also evaluated.

Methods: SULF1 expression in 112 invasive ductal breast cancer samples was detected by immunohistochemistry. The relationship between SULF1 expression level with clinicpathological variables and overall survival was analyzed.

Results: SULF1 staining was located in both cancer cells and stromal components. SULF1 expression in stroma was significantly inversely associated with lymph-node metastasis (P=0.020), late TNM stage (P=0.032), ER status (P=0.034), and triple-negative phenotype (P<0.001). SULF1 expression in cancer cells was also found to be inversely related to lymphnode metastasis (P=0.007), PR status (P=0.016) and triple-negative phenotype (P<0.001). Furthermore, stromal SULF1 expression, but not cancerous SULF expression was significantly associated with a better prognosis, but also emerge as an independent predictor for breast cancer.

Conclusion: Our data supported that SULF1 acts as a negative regulator during breast carcinogenesis, and we suggest that SULF1 in the stromal cells might also participate in the modulating of heparin-binding growth factors and cytokines as it acts in cancer cells.

Key words: SULF1, breast cancer, prognosis, immunohistochemistry.

Introduction

Breast cancer ranks the first causes of cancer-related death in women over the world. Even received standard treatment involving surgery, chemotherapy, radiation, endocrine therapy, and targeted therapy, there are still a proportion of therapeutic failures which result in cancer recurrence, metastasis and death. Therefore, there is an urgent need for novel specific prognostic factors or potential therapeutic targets for breast cancer patients.

Heparan sulfate proteoglycans (HSPGs) act as co-receptors for heparin-binding growth factors and cytokines on the cell surface and in the extracellular matrix ¹. Sulfatase 1 (SULF1), one of HSPG members, selectively removes 6-O-sulfate groups from heparan sulfate, which could alter its binding sites for growth factors and cytokines. The important roles of SULF1 in carcinogenesis have been emphasized in previous reports; however, these results are controversial. Some reports support SULF1 as a negative regulator of cancer cell growth, which might be associated with its deregulating effect on several factors, such as FGF2, HB-EGF, and HGF². Meanwhile, some other studies indicated that SULF1 as a positive regulator of oncogenic signaling pathways, such as Wnt, BMP, Hedgehog, and GDNF^{3,4}.

The roles of SULF1 in breast cancers have also been unequivocally highlighted. For example, it has been reported that hypermethylation status in the promoter region of SULF1 gene correlated with the down-regulation of the SULF1 expression in human breast cancer cell lines and tissue samples, furthermore, SULF1 promoter hypermethylation was more frequently in serum cellfree DNA samples of human breast cancer patients than the healthy controls ⁵. In contrast, in a metaanalysis on gene expression data from 44 datasets, Bret et al. ⁶ found that SULF1 was over-expressed in human breast cancers in comparison with their normal counterparts. Currently, the expression of SULF1 in breast cancers at protein level has been relatively poorly understood, therefore, in this study, we immunohistochemically investigated the expression of SULF1 in a cohort of breast cancer tissues, and its clinicopathological and prognostic significance was also evaluated.

Materials and Methods

Patients

A total of 112 primary breast invasive ductal carcinomas were retrospectively investigated in this study. All of the patients received curative surgery from January 1998 and October 2009. None of the patients in the study received preoperative chemotherapy. The follow-up time ranged from 11 to 131 months. Overall survival time was defined as the time from operation to cancer-related death only. The study was approved by the research board of our institutions. The clinicaopathological characteristics of patients included in this study were summarized in table 2.

Immunohistochemistry

Immunohistochemistry was performed on 4 μ m thickness of formalin-fixed, paraffin-embedded breast cancer sections. Immunostaining was detected with the Rabbit polyclonal antibody to SULF1 (Abcam dilution 1: 100). For the negative controls, the primary antibodies were replaced with Rabbit IgG (Abcam) of the same concentrations. The results were interpreted in cancerous and stromal counterparts for each sample, respectively. If staining was observed in more than 10% of the cancer cells or stromal fibroblasts, the staining was defined as cancerous-positive or stromal-positive. The scor-

ing for immunostaining was performed independently by two pathologists who were blinded to the corresponding clinical information.

Statistics

The comparisons of clinicopathologic variables between different subgroups were made using χ^2 test or Student's t test when appropriate. Overall survival of breast cancer patients were estimated by the Kaplan-Meier method, differences were compared by the log-rank test. Multivariate analysis was performed using a Cox proportional hazard model. P<0.05 was considered statistically significant. All calculations were done by the SPSS 15.0 package.

Results

We examined the SULF1 protein expression in 112 invasive breast cancer tissues by immunohistochemistry. We observed significant staining of SULF1 in both stromal and cancerous cells (Figure 1). According to the criteria established, 67.9% (76/112) cases were defined as positive cancerous expression, while 62.5% (70/112) were defined as positive stromal expression. SULF1 staining was located in the cytoplasm and membrane in cancer cells and in the cytoplasm of stromal fibroblasts.

Next, we analyzed SULF1 expression with various clinicopathological variabes (Table 1).



Figure 1. Protein expression pattern of SULF1 in breast cancer tissues. Representative images of SULF1 expression in stromal (A) and cancer cells (B), respectively. Original magnification 200×.

| | | Cancerous SU | LF-1 staining | | Stromal SUI | LF-1 staining | |
|----------------------|-------|--------------------|--------------------|---------|--------------------|--------------------|---------|
| | Total | positive (n=76) | negative (n=36) | P value | Positive (n=70) | Negative (n=42) | P value |
| Age (year) | | 51.2±11.1 | 56.0±14.4 | 0.053 | 51.2±10.9 | 55.2±14.4 | 0.102 |
| Grade | | | | | | | |
| 1 | 37 | 27 | 10 | | 24 | 13 | |
| 2 | 71 | 47 | 24 | 0.572 | 44 | 27 | 0.834 |
| 3 | 4 | 2 | 2 | | 2 | 2 | |
| Tumor size | | | | | | | |
| T1 | 23 | 13 | 10 | | 13 | 10 | |
| T2 | 78 | 55 | 23 | 0.422 | 50 | 28 | 0.802 |
| T3 | 11 | 8 | 3 | | 7 | 4 | |
| Lymphnode metastasis | | | | | | | |
| Negative | 42 | 35 | 7 | 0.007 | 32 | 10 | 0.020 |
| Positive | 70 | 41 | 29 | | 38 | 32 | |
| TNM stage | | | | | | | |
| Ι | 6 | 4 | 2 | | 5 | 1 | |
| Π | 69 | 50 | 19 | 0.392 | 48 | 21 | 0.032 |
| III | 37 | 22 | 15 | | 17 | 20 | |
| ER | | | | | | | |
| Negative | 42 | 24 | 18 | 0.06 | 21 | 21 | 0.034 |
| Positive | 70 | 52 | 18 | | 49 | 21 | |
| PR | | | | | | | |
| Negative | 50 | 28 | 22 | 0.016 | 28 | 22 | 0.202 |
| Positive | 62 | 48 | 14 | | 42 | 20 | |
| Her2 | | | | | | | |
| Negative | 83 | 56 | 27 | 0.882 | 52 | 31 | 0.956 |
| Positive | 29 | 20 | 9 | | 18 | 11 | |
| Phenotype | | | | | | | |
| Non triple-negative | 91 | 68 | 23 | 0.001 | 64 | 27 | < 0.001 |
| Triple-negative | 21 | 8 | 13 | | 6 | 15 | |

Table 1. Association of SULF-1 with clinicopathological characteristics in breast carcinomas

 Table 2. Multivariate analysis for overall survival

| Characteristics | Sia | Evr (D) | 95.0% CI for Exp(B) | | |
|--------------------------|-------|---------|---------------------|-------|--|
| Characteristics | 51g. | Ехр(Б) | Lower | Upper | |
| Age | 0.696 | 1.006 | 0.976 | 1.038 | |
| Grade | 0.526 | 1.270 | 0.606 | 2.661 | |
| Tumor size | 0.313 | 1.525 | 0.671 | 3.465 | |
| Lymphnode metastasis | 0.811 | 0.875 | 0.293 | 2.614 | |
| Stage | 0.248 | 1.835 | 0.655 | 5.141 | |
| ER | 0.994 | 0.994 | 0.224 | 4.422 | |
| PR | 0.120 | 0.405 | 0.130 | 1.266 | |
| Her2 | 0.426 | 0.566 | 0.139 | 2.300 | |
| Triple-negative | 0.818 | 1.238 | 0.200 | 7.652 | |
| Negative stromal SULF1 | 0.038 | 3.015 | 1.064 | 8.544 | |
| Negative cancerous SULF1 | 0.652 | 0.797 | 0.296 | 2.141 | |



Figure 2. Kaplan-Meier analysis in breast patients according to the expression of SULF1 in stroma (A) (P < 0.001) and in cancer cells (B) (P = 0.07)

SULF1 expression in stroma was significantly inversely associated with some factors reflecting disease progression, such as positive lymph-node metastasis (P=0.020), late TNM stage (P=0.032), negative ER status (P=0.034), and triple-negative phenotype (P<0.001). Furthermore, SULF1 expression in cancer cells was also found to be related to non-lymphnode metastasis (P=0.007), negative PR status (P=0.016) and non-triple-negative phenotype (P<0.001).

To further investigate the significance of SULF1 in terms of overall survival, we performed Kaplan-Meier analysis (Figures 2A and 2B). The overall survival rates were significantly higher in the stromal SULF1-positive subgroup than the negative subgroup (P<0.001). Cancerous SULF1 expression also showed a negative association with shorter overall survival, but a statistical significance has not reached (P=0.07). Furthermore, in multivariate Cox proportional hazards regression analysis, negative stromal SULF1 expression emerged as an independent prognostic factor for the survival of patients with breast cancer (p=0.038) (table 2).

Discussion

In this study, we identified SULF1 protein significantly up-regulated in a proportion of breast cancers. We identified both cancerous and stromal expression pattern in breast cancer tissues. Our data revealed that both cancerous and stromal SULF1 expression is inversely associated with a more aggressive and progressive tumor phenotype. Furthermore, stromal SULF1 expression, but not cancerous SULF expression was associated with a better prognosis, but also retained an independent predictor for breast cancer.

Our results supported that SULF1 acts as a negative regulator during breast carcinogenesis, which is consistent with the data of most previous in vitro studies. Narita et al.7 indicated that SULF1 could modulate the function of heparan sulfate binding VEGF165 in proliferation and angiogenesis in MDA-MB-468 breast carcinoma clonal lines, and inhibited angiogenesis and tumorigenesis in vivo. They further found that SULF1 inhibited autocrine activation of the EGFR-ERK pathway in MDA-MB-468 breast cancer cells⁸. Recently, Khurana A, et al.9 indicated that loss of SULF1 under hypoxic microenvironment was associated with increased growth factor signaling, cell migration, and invasion in breast cancer cells. In particular, high SULF1 mRNA expression by in situ hybridization is associated with increased overall survival9, which is consistent with our findings at protein level by immunohistochemistry.

It has been demonstrated cancer-related fibroblasts differ functionally with their normal controls in various solid malignancies including breast cancer¹⁰. Emerging evidence support the notion that stromal components of breast cancer tissues could play important roles in tumor

initiation, progression, and metastasis¹¹. Several stromal markers have been identified be over-expressed in breast cancer tissues¹². Our data indicated that similar with cancerous expression, stromal SULF1 expression inversely correlated with progression phenotype and poor prognosis. Stromal fibroblasts of breast cancer tissues are also rich sources of heparin-binding growth factors and cytokines such as FGF2, HGF¹⁰, therefore, we proposed that SULF1 in stromal cells might act in a similar fashion as in cancer cells. Elevated stromal SULF1 expression had also been identified in gastric cancer¹³. However, different with our data, the expression of stromal SULF1 protein in gastric cancer was positively associated with a more aggressive tumor phenotype and worse prognosis. This discrepancy may be due to differences in the stromal fibroblasts in different cancer types.

In conclusion, in this study, we found upregualted SULF1 was located not only in cancer cells, but also in stromal counterpart of breast cancer tissues. Both cancerous and stromal SULF1 expression was inversely associated with a more aggressive and progressive tumor phenotype. Furthermore, stromal SULF1 expression predicted better prognosis for patients with breast cancer, and was also an independent prognostic factor.

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Lichtenstein technique - relapses and postoperative chronic pains

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Abstract

Introduction: The Lichtenstein technique has been set as the golden standard in good practice guides for hernia surgeries and it has revolutionized hernia repairs. The incidence of relapses has been very much reduced, postoperative pain is much lesser and lasts for shorter periods of time, hospitalization lasts for only a few hours to just one day, and reverting to normal life activities and work is very quick. A relapse is recurrence of a hernia at the place of a former surgery while inguinodynia is a chronic postoperative pain in the groin that has been operated on.

Objective of the paper: Is to determine incidences of relapses and inguinodynias, i.e. chronic pains that occur after hernia repairs according to Lichtenstein, and to point to risk factors that cause occurrence of postoperative complications.

Materials and methods: The study is of retrospective-prospective character and covers 1460 patients operated on for inguinal hernia in the Medical System Belgrade. Out of that number, 1164 patients were operated on according to the Lichtenstein method and 296 by applying one of 'tension' techniques. The study was conducted as prospective in the period from 01/01/2008 to 31/12/2012 and, as retrospective, in the period from 01/01/2003 to 31/12/2007. In that period, in the Medical System Belgrade, 4380 patients were surgically treated. The study covers patients operated on for inguinal hernia, either direct or indirect, while the data on femoral and incisional ones were not processed. The data from case histories were used and, in patients who were operated on in the prospective part of the study, in addition to case histories, questionnaires were used, which were filled in by patients at admission. Patients were classified according to the localization of hernias and according to the type of surgical technique that was applied on inguinal hernias. In addition,

case histories were used and data on follow-ups that are performed after 15 days, one, three, and six months, and sometimes even subsequently.

Results: Out of the total number of those operated on for inguinal hernias (1420), 1164 (79.7%) of the patients were operated on according to the Lichtenstein method. In the 2003-2005 period, 'tension' (208, i.e. 46%) and tension-free surgical techniques (244, i.e. 56%) were almost equally applied and, in the 2006-2012 period, tension-free surgical techniques were most frequently applied, specifically in 95% of the cases (920) as opposed to 'tension' ones, which were performed in only 5% (48) of the cases. The incidence of relapses in the study period amounts to 2.8%, and the incidence of inguinodynias, i.e. pains that lasted longer than for one year, amounts to 2.5%. The total number of relapses in 'tension' techniques accounts for 8.98% (23/256) and the number of relapses in the Lichtenstein technique amounts to 1.012% (13/1164). Out of the total number of patients operated on for hernias, we had 11 (0.77%)patients, who experienced chronic pain, which occurred after the surgery and lasted longer than a year. Out of that number, six (2.34%) patients were operated on by applying 'tension' techniques and five (0.43%) by applying the method according to Lichtenstein.

Conclusions: The incidence of relapses was reduced after the introduction of the Lichtenstein technique into hernia repairs, same as the incidence of inguinodynias.

Key words: Hernia, Lichtenstein technique, relapse, inguinodynia.

Introduction

Hernia repairs are the most frequent surgeries in general surgery and, according to some studies, in surgery in general as well. We can classify all the currently known techniques into 'tension', tension-free, and laparoscopic ones. Each of them has its application and target group, but the commonest technique of hernia repairs in the world is the one according to the Lichtenstein method (1).

The conventional suture technique (non-mesh one), the first rational technique, was described by Bassini in 1884, but unfortunately his original surgery has been modified and abused. Such situation had lasted until 1950, when the modern version of the original Bassini surgery was described by Shouldice in which the posterior wall of the inguinal canal and the internal ring are reconstructed in several layers using continuous non-resorptive, monofilament suture (2).

Recent randomized studies have shown that the Shouldice technique is considerably better than the non-original Bassini technique and the Marcy one (simple narrowing of the inguinal ring) with the percentage of relapses of 15.33 and 34% during a long-term follow-up. Therefore, the Bassini technique and the Marcy technique are obsolete. The Shouldice technique is the best conventional treatment for primary inguinal hernias. In experienced hands, results are very good (the relapse rate of 0.7-1.7%). In general practice, results are less satisfactory with the relapse rate of 1.7-15% during a long-term follow-up (3).

Techniques with mesh

Approximation of tissues that normally do not lie in juxtaposition results in an abnormal tension between such tissues. All the conventional suture techniques of inguinal hernia repairs share this factor - tension on sutures. This may result in ischemia, which is the source of pain, necrosis, rupture on sutures, and hernia relapses. It is known that some patients suffering from inguinal hernias have abnormal metabolism of collagen, particularly the older ones. Strengthening of such tissues by synthetic materials is the established method. The concept of tension-free reconstruction of the defect was established by the end of the 19th century, but adequate biomaterial in the form of polypropylene meshes became available in 1960. Mesh material, which is nowadays mostly used, is a flat sheet of polypropylene.

Prosthetic reconstruction of the defect of the posterior wall of the inguinal canal can be performed in two fundamentally different ways. The defect is blocked by a plug, or the mesh prosthesis is positioned over the transversalis fascia. The prosthesis can be positioned in the groin frontally, through an inguinal incision, or posteriorly into the preperitoneal space, through a conventional open access or endoscopically. The frontal open tension-free reconstruction of inguinal hernias was promoted by Lichtenstein in 1984. Through an inguinal incision, a polypropylene mesh is stitched to the posterior wall of the inguinal canal with significant overlaying. The mesh is positioned between the internal oblique muscle and aponeurosis of the external oblique muscle and is stitched to the inguinal ligament. The key is adequate overlying of the posterior wall of the inguinal canal, specifically 2 cm medially from the tuberculum pubicum.

The results from literature, covering techniques of reconstruction of inguinal hernias, corroborate that the Shouldice technique is the best non-mesh reconstruction for primary inguinal hernias. The Lichtenstein technique, introduced in 1984, is currently the best verified and the most popular one among different open mesh techniques. It can be performed with minimum perioperative morbidity, as one-day surgery (under local anesthesia), and has a low relapse rate ($\leq 4\%$) during a longterm follow-up (4).

Lichtenstein surgical technique

Make an incision sufficiently medially for a good exposition of the tuberculum pubicum and the rectus body. Ligate superficial veins. Enter into the cleavage of the externus obliquus (preserve the ilioinguinal nerve). Make a loop around the funiculus spermaticus. Approach the posterior wall. The cremaster does not have to be excised, unless it is hypertrophic, and then leave the extended internal ring. Prepare the hernia sac up to the inside of the internal ring and resect or reduce or reposit it. If necessary, suture a large direct hernia without tension continuously applying resorptive suture so that a flat posterior wall with a normal internal ring is formed.

In principle, all the nerves should be preserved, but they should be cut without hesitation if they are damaged or interfere with the mesh. Particular attention should be devoted to the iliohypogastric nerve; it may lie underneath the mesh, but it is desirable that it is not in juxtaposition with the sharp edge (cut the prosthesis down to the desired size; separation of the nerve is better than causing a neuralgic pain).

Position the polypropylene 7 x 14 cm mesh (curbing is often required) with overlaying of 2 cm over the tuberculum pubicum. Suture continuously using polypropylene 3-0 starting at 2 cm mediocranially to the lateral edge of the rectus and then to the inguinal ligament up to the internal ring. Make an incision at 1/3 of the lower side all the way up to directly underneath the funiculus. Suture both tails of the prosthesis overlaying the lateral side of the inguinal ligament using one polypropylene suture; the upper tail over the lower one. Fix the cranial edge of the mesh using one or a number of stitches (which may be resorptive sutures) to the aponeurosis of the internus obliquus avoiding the muscle in order to avoid injury of the intramuscular part of the iliohypogastric nerve. Be careful to avoid making a loop around the nerves during suturing. The mesh must lie without tension (conically) after removal of ecarteur. After that, suture the aponeurosis externus and thereafter the subdermis and skin (5).

Chronic pain

From a general medical aspect, a pain that lasts for three months after surgery, while resting or under strain, is defined as chronic, while herniologists deem that a chronic pain is the one that persists for a year after surgery. Regardless of the fact that the incidence of chronic postoperative pains - both in mesh surgeries and in non-mesh ones - is less than 1 %, a chronic pain is considered to be one of the biggest complications of hernia repairs. In 2009, the European Hernia Association, for this reason, recommended that small and asymptomatic hernias should not be operated on immediately but should be just observed and carefully followed up, because of the assessment that the chances of occurrence of chronic pain are bigger than of jamming of 'small' inguinal hernias.

It has also been noticed that a chronic pain more frequently occurs in patients who had had experienced pain even before surgery, as well as in younger population. In the west, there is also the controversy related to the postoperative experience of chronic pain, because it is most frequently reported by manual workers, who thereafter apply for remuneration of the costs of surgery. However, the fact is that there are cases of severe disability caused by chronic pain, i.e. persons who had lived with hernias almost without any problems, but the postoperative chronic pain very much limits them in everyday life and work.

Chronic pain is a disease in its own right, which must be treated in a multidisciplinary way, by psychiatrists, psychologists, physiotherapists, by applying acupuncture, antirheumatic drugs, and local anesthesia in the wound. In case of a failure, even a surgical solution of chronic pain is attempted, which implies extraction of the implant and, as the last resort, even cutting of (all) the three nerves responsible for the pain in the groin – the so-called triple neurectomy, which is successful in around 40 % of the cases.

All the experts in this area agree that treatment of a chronic pain should be reserved for hernia experts, because the chances of full recovery are rapidly diminished after every repeated surgery (6).

Objective of the paper

Several objectives were set in this paper. Objectives of this paper are to:

- Determine incidence of relapses after hernia repairs according to Lichtenstein
- Determine incidence of inguinodynia, i.e. chronic pain that occurs after hernia repairs, according to Lichtenstein, and
- Point to risk factors that cause occurrence of postoperative complications.

Materials and methods

The study is of retrospective-prospective character, covering 1420 patients operated on for inguinal hernia in the Medical System Belgrade. The study was conducted as prospective in the period from 01/01/2008 to 31/12/2012 and, as retrospective, in the period from 01/01/2003 to 31/12/2007.

In that period, in the Medical System Belgrade, 4380 patients were surgically treated. The study covered patients operated on for inguinal hernia, either direct or indirect, while the data on femoral and incisional ones were not processed.

The data were used from case histories and, in patients who were operated on in the prospective

part of the study, in addition to case histories, questionnaires were used, which were filled in by the patients at admission.

Patients were classified according to the localization of hernias, according to the type of inguinal hernias, and according to the type of surgical technique that was applied on inguinal hernias. The data were processed applying modern methods of descriptive and analytical statistics. The results are shown in tabular form and graphically.

The questionnaire, which was drawn up for the purpose of the prospective part of the study:

Name and family name Place and year of birth Have you had any diseases and which ones?..... _____ Do you suffer from diabetes (if yes, since when)? Do you suffer from asthma (if yes, since when)?.... Have you undergone any surgeries (which ones and when)?..... Since when have you had hernia? _____ How did it develop (suddenly or gradually)? Has it been jammed and how many times? Can the hernia you have got be reduced or it is always present without the possibility of reduction? Is there anyone with a hernia in the family? _____ Have you been operated on for hernia before? _____ When were you operated on and were you operated on on the same side and have you hernia now? Who was the surgeon that operated on you? Roughly, how old was your surgeon? Did you have an infection or suggillation in the wound then?

| On what postoperative d | ay did you l | leave the hos- |
|-------------------------|--------------|----------------|
| pital? | | |

Results

In the study period, there were 35.6% of those operated on for hernias of different localizations. *Table 1. Number of those operated on in the 2003-2012 period*

| Type of surgery | Incidence | % |
|------------------|-----------|------|
| Hernia surgeries | 1560 | 35.6 |
| Other surgeries | 2820 | 64.4 |
| Total surgeries | 4380 | 100 |

The most frequent hernias by the localization were inguinal hernias, with 91%.

Table 2. Number of those operated on for hernias by the localization of hernias in the 2003-2012 period

| Localization of hernias | Number of those operated on | % |
|----------------------------|--------------------------------|------|
| Inguinal | 1420 | 91.0 |
| Femoral | 38 | 2.40 |
| Umbilical | 62 | 4.00 |
| Epigastric | 21 | 1.35 |
| Incisional | 19 | 1.25 |
| Total hernias | 1560 | 100 |

In the 2003-2005 period, both 'tension' and tension-free surgical techniques were almost identically applied in hernia repairs.

Table 3. Share of surgical techniques in inguinal hernias in the 2003-2005 period

| Type of surgical technique | Number of those operated on | % |
|----------------------------|--------------------------------|-----|
| 'Tension' techniques | 208 | 46 |
| Tension-free techniques | 244 | 54 |
| Total surgeries | 452 | 100 |

In the 2006-2012 period, tension-free techniques were applied in 95% of the patients operated on for inguinal hernias.

Table 4. Share of surgical techniques in inguinal hernias in the 2006-2012 period

| Type of surgical technique | Number of those operated on | % |
|----------------------------|--------------------------------|-----|
| Tension-free techniques | 920 | 95 |
| 'Tension' techniques | 48 | 5 |
| Total surgeries | 968 | 100 |

| Table 5. | Share of surgical techniques | in inguinal |
|-----------|------------------------------|-------------|
| hernias i | n the 2003-2012 period | |

| Type of technique | Number of those operated | % |
|-------------------|-----------------------------|------|
| Tension-free | 1164 | 81.9 |
| 'Tension' | 256 | 18.1 |
| Total surgeries | 1420 | |

The total number of relapses after inguinal hernia repairs applying 'tension' surgical techniques accounts for 8.98% (23/256).



Diagram 1. The incidence of relapses in 'tension' surgical techniques

The total number of relapses after inguinal hernia repairs by applying the Lichtenstein surgical technique accounts for 1.12% (13/1164).



Diagram 2. The incidence of relapses in the Lichtenstein surgical technique

Out of the total number of patients operated on for hernias, we had 11 (0.77%) patients, who experienced a chronic pain that occurred after surgery and lasted for over a year. Out of the total number of patients suffering from a chronic pain, six of them were operated on by applying 'tension' techniques and five of them by applying the technique according to Lichtenstein.



Diagram 3. The incidence of chronic pains in those operated on for hernias

Discussion

In the study period, there were 35.6% of those operated on for hernias of different localizations, which constitutes one third of all those operated on in the study period and it is a very frequent operation in surgery. The most frequent hernias by localization were inguinal hernias, with 91%. According to the data from literature, the total incidence of occurrences of inguinal hernias in adult population varies from 10 to 15%. The ratio between men and women is 12: 1. In the USA, over 700,000 surgeries of inguinal hernias are performed annually and it is the most frequently performed surgical procedure in the USA (1).

In the 2003-2005 period, both 'tension' and tension-free surgical techniques were almost equally applied in hernia repairs but, in the 2006-2012 period, tension-free techniques were applied in 95% of the cases, which gave rise to a significant progress in the surgical technique and improvement of the outcome of surgical interventions in many patients. The total number of relapses after inguinal hernia repairs by applying 'tension' surgical techniques accounted for 8.98% (23/256), while the total number of relapses after inguinal hernia repairs by applying the Lichtenstein surgical technique amounted to 1.12% (13/1164), which corresponds to the data from literature. According to the literature, tensionfree techniques have become the "golden standard" in repairing of the defect of the abdominal wall and the indicative population for conventional - 'tension' procedures includes younger patients suffering from smaller inguinal (both direct and indirect) and femoral hernias (4). Out of the total number of patients operated on for hernias, we had 11 (0.77%)patients suffering from chronic pain that occurred after the surgery and lasted for over a year. Out of the total number of patients experiencing chronic pain, six of them were operated on by applying

'tension' techniques and five by applying the technique according to Lichtenstein, which corresponds to the data from literature (6).

Conclusions

The incidence of relapses after inguinal hernia repairs, when the surgical technique according to Lichtenstein is applied, is significantly lower than when applying 'tension' techniques.

The incidence of inguinodynias is significantly lower after applying the Lichtenstein surgical technique than after applying 'tension' techniques.

There are numerous risk factors that contribute to the occurrence of relapses after hernia repairs, and our study points to the fact that the selection of the surgical technique is the factor of particular importance.

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Methylation of Epstein-Barr virus-associated gastric cancer suppressor genes

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Abstract

Objective: To identify the methylation silenced tumor suppressor genes in Epstein-Barr virus (EBV)-associated gastric cancer (EBVaGC). Methods: EBV-positive (GT38, PT and SNU719) and negative (SGC7901) gastric cancer cell lines were selected and treated with 5-Aza-CdR. Then real-time fluorescence quantitative PCR was used to validate the results of microarray, and methylation-specific PCR (MSP) and bisulfite genomic sequencing (BGS) were adopted to detect the CpG island methylation levels of gene promoters.

Results: The expression levels of 6 differentially expressed genes (H19, LOXL1, ARMCX2, LXN, CDH3 and MMP7) before and after 5-Aza-CdR treatment were confirmed by real-time qPCR, which are consistent with the results of microarray analysis. There were different degrees of methylation in LOXL1 gene promoter in EBVaGC. GT38 and PT were fully methylated, and SGC7901 and HGC-27 was unmethylated, suggesting that this gene is a candidate methylation silenced tumor suppressor gene. The methylation rate of LOXL1 in EBVaGC was significantly higher than that in EBV-negative gastric cancer (EBVnGC).

Conclusion: The promoter region of candidate tumor suppressor gene LOXL1 shows high methylation status, indicating that EBV critically accounts for the methylation of LOXL1 gene regulatory region. EBV is involved in the pathogensis of EBVaGC that aberrant methylation occurred in promoter CpG island, which inactivates tumor suppressor genes.

Key words: Epstein-Barr virus, gastric cancer, methylation, tumor suppressor gene

Introduction

Epstein-Barr virus (EBV), the strain of which was successfully established by Epstein and Barr for the first time in 1964 by the in vitro suspension culture of Burkitt African children lymphoma cells, leads to a variety of malignancies (e.g. nasopharyngeal carcinoma) [1]. EBV mainly infects B lymphocytes manifested with non-proliferation infection [2], and stratified squamous epithelial cells accompanied by viral replication inducing B cellderived tumors and epithelial cell-derived tumors [3]. The disease is widely distributed worldwide and may be epidemic because more than 90% of healthy adults are carrying this virus [4]. The onset ages range mostly between 15 and 30 years old, and children below 6 often show inapparent infection. EBV-related diseases occur throughout the year, which seems to peak in late autumn and early winter. Once infected, the patients usually get relatively long-lasting immunity. The relationship between EBV infection and gastric cancer was confirmed in the 1990s. The EBV infection rate of lymphoepithelioma-like gastric cancer is up to 80% to 100%, and there that of ordinary gastric cancer differs regionally by about 10% [5]. DNA methylation is the most cutting-edge epigenetic mechanism, in which the role of methylationbased epigenetic change in tumorigenesis is of the greatest concern. In addition to mutation and deletion, the hypermethylation of tumor suppressor gene promoter is considered to dominate the inactivation of tumor suppressor gene, which plays an important role in the occurrence and development of tumor [6]. About 20% of human tumors are related to viral infections. The abnormal epigenetics of host cell caused by virus infections has been spotlighted in the current research on the carcinogenic mechanism of tumor virus [7].

Studies have shown that hypermethylation of some tumor-related genes [8] (tumor suppressor gene, apoptosis-related gene and mismatch repair gene, etc.) exists in EBV-associated gastric cancer (EBVaGC), whose methylation frequency and methylation density of promoter CpG island are higher than those of EBV-negative gastric cancer (EBVnGC). High methylation is negatively correlated with the expression of tumor suppressor genes in EBVaGC, such as p16 and p73. EBV infection induces aberrant methylation in CpG island that inactivates cancer suppressor genes, which may be the mechanism of EBV involved in the occurrence of EBVaGC. However, the relationship between EBV genome methylation and host cell gene methylation, as well as how EBV induces the methylation of suppression cancer genes of host cell have not been clarified yet [9].

EBV-positive and-negative gastric cancer cell lines were selected as the object in this study, which were treated with 5-Aza-CdR [10] to induce the reexpression of methylation silenced tumor suppressor genes, and an untreated control group was established simultaneously. The methylation statuses of target genes in EBVaGC and EBVnGC were detected and compared, and the relationship between the methylation of cancer suppressor genes and clinical-pathological characteristics were further analyzed and identified define EBVaGC-specific apparent molecular genetic marker.

Materials and Methods

Apparatus

The apparatus included PTC-100 PCR, ABI-7000 quantitative fluorescence PCR (ABI), spectrophotometer, high-speed low-temperature centrifuge (Eppendor), CO_2 incubator (SHEIL-LAB), G2545A hybrid reactor, G2565BA microarray scanner (Agilent), inverted microscope, glass tube (NarishigeGD-1 core tube, 1×90 mm core-free tube), gel imaging system, ultra0low-temperature refrigerator, and constant-temperature water bath.

Reagents and materials

EBV cells were stored in our laboratory. DMEM culture medium, TRIzol and fetal bovine serum were purchased from GIBCO. 5-aza-deoxycytidine was purchased from Sigma. Microarray hybridization reagents were bought from Agilent. The other reagents and materials include AM8436 aaUTP (Alnbion), 0.2 ml PCR reaction tube, 15 ml centrifuge tube, 1 ml and 1.5 ml Eppendor tubes, PCR reagents (dNTp, MgCl₂, 10×buffer), trypsin, agarose and DNA marker DL-2000.

Cell culture and treatment

DMEM medium was used to cultivate 4 cell lines (GT38, PT, SNU719 and SGC7901), subcultured once every 2 to 3 days. 5 mg was then taken and fully dissolved with DMEM medium, allowing the constant volume to 43.8 mL, which was prepared into a 500 µmol/L mother liquor and stored at -20°C. Then it was diluted to a 10 µmol/L working solution by DMEM medium. The cells in logarithmic growth phase were sampled. When the cell convergence concentration reached 70%--80%, the medium was abandoned and the cells were washed twice with PBS. DMEM medium of 5-Aza-CdR containing 10 µmol/L methylation inhibitor was added, and the group without drug was used as the control. The total cellular RNA was extracted after 4 continous days.

Differentially expressed genes identified by Real-time qPCR

The total cellular RNA was extracted for RNA inverse transcription according to the standard conditions required by reverse transcription kit. cDNA obtained from inverse transcription was used as the template for PCR reaction and stored at -20°C. Amplification and data analysis were conducted on a LightCycler480 II fluorescent quantitative PCR instrument with the SYBR Green I dye method. The result data of Real- time qPCR were analyzed to determine the Ct value of each group. Ct value represents the number of cycles experienced when fluorescence signal in each reaction tube reached the specified threshold. Ct value of each template is linearly correlated with the logarithm of initial copy number of the template. Δ Ct value of each sample was analyzed with the relative quantitative method and \triangle Ct=target gene Ct - internal reference gene Ct. 2^{-ΔΔCt} was calculated according to $\triangle \Delta Ct$ =treatment group ΔCt - control group ΔCt . When the amplification efficiencies of target and internal reference genes are close, 2-AACt represents the times of expression of target gene in the sample in the treatment group relative to that in the control group (Table 1).

DNA methylation

Fresh gastric cancer and corresponding pericarcinomatous tissues resected from 975 gastric cancer patients in our hospital were collected. The

| Gene | Туре | Sequence | Annealing temperature (°C) | Length (bp) |
|----------|----------|---------------------------|----------------------------|-------------|
| 1110 | Primer 1 | GGCCTTCCTGAACACCTTAG | 56 | 1.42 |
| П19 | Primer 2 | TGAGCTGGGTAGCACCATTT | 50 | 142 |
| ADMCV2 | Primer 1 | CTGCTTGTCAATTCCATTGC | 59 | 142 |
| ARIVICAZ | Primer 2 | CTGGCACTTGGGTACTGAGA | 30 | 142 |
| LOVI 1 | Primer 1 | ACTGCCAGTGGATCGACATA | 50 | 101 |
| LUALI | Primer 2 | ATGTTGCATCTAACCACGTT | | 121 |
| IVN | Primer 1 | GGAGATTCCCTGGCAAA | 59 | 01 |
| LAN | Primer 2 | TTTGGCACACGGCTATTATG | 38 | 01 |
| | Primer 1 | GACATCATGATTGGCTTTGC | 59 | 120 |
| | Primer 2 | TCCTCATCGAAGTGAGCATC | 56 | 120 |
| CDU2 | Primer 1 | AGATCACCATCTGCAACCAA | 50 | 146 |
| CDR3 | Primer 2 | GTCACCTTCCTCGTTGACCT | | 140 |
| GAPDH | Primer 1 | CCTAGACACACCATGGGGAAGGTGA | | 450 |
| | Primer 2 | ATGATCTTGAGGCTGTTGTCATA | | 430 |

Table 1. Primer sequences of real-time qPCR detection

| Table 2. | Methy | lation-spe | cific PCR | and sulfite s | salt-gene | e sequencing | g primers |
|----------|-------|------------|-----------|---------------|-----------|--------------|---------------|
| | | | | | | | Annoaling tan |

| Gene | Туре | Sequence | Site | Annealing temperature (°C) | Length (bp) |
|-------|------|---------------------------|-----------|-------------------------------|----------------|
| | M1 | TTAGTAGGCGGATTAGGAGTC | 12 125 | 60 | 179 |
| | M2 | ACGATACCCAAAACTACACGAA | -43~133 | 00 | 1/0 |
| | U1 | AGTAGGTGGATTAGGAGGTTGA | 41 127 | 59 | 179 |
| LUALI | U2 | AAACAATACCCAAAACTACACAAA | -41~137 | 58 | 1/0 |
| | 1 | TTTTAGGGATTGAGGGAGTATTGTA | -126~-101 | 60 | 274 |
| | 2 | AAAAACTCCTTACTTACCCCAACTC | 223~248 | 00 | 5/4 |

tumor was taken immediately after being separated from the body under aseptic conditions. A tissue block with the size of 1 cm³ was taken from the non-necrotic tumor tissue, placed in a sterile bottle and treated as soon as possible or stored at -80°C. Tissue DNA was routinely extracted with the phenol-chloroform-isoamyl alcohol method. Meanwhile, part of tissues were taken from each sample, fixed with PBS-10% formalin and embedded with paraffin to prepare tissue sections for pathological diagnosis and EBER in-situ hybridization to screen EBVaGC. 23 cases of EBVaGC sample with strong EBER in-situ hybridization signal and high quality DNA were selected as the positive EBV group and 22 cases of EBVnGC sample were rendered as the negative ENV group.

Nucleic acid was extracted from fresh gastric cancer tissue and corresponding pericarcinomatous tissue cells, and the extracted DNA was modified and purified by nitrate. MSP was conducted on EBV positive gastric cancer cell lines modified by utilizing hydrosulfite, EBV negative gastric cancer cell lines and tissue DNA as the templates. The methylation-specific primers, non-methylation-specific primers and the sizes of MSP products are shown in Table 2. To further detect the specific methylation status at CpG locus in the promoter region of LOX-LI coding gene and verify the MSP results, primer line BGS sequences outside MSP primer sequence were designed online using MethPrimer. The results were recorded by a UV gel imaging system.

Statistical analysis

The gene promoter methylation detection data were analyzed by PPMS1.5. The measurement data were compared by the calibration test. P=0.5 was considered statistically significant.

Results

Differentially expressed genes identified by Real-time qPCR

To verify the differential genes identified by gene chip analysis and preliminarily screen methylati-

| Gene | GT38 | | РТ | | SUN719 | | SCG7910 | |
|--------|------|-------|-------|-------|--------|-------|---------|-------|
| | qPCR | chip | qPCR | chip | qPCR | chip | qPCR | chip |
| H19 | 6.32 | 18.17 | 3.25 | 9.15 | 4.76 | 24.50 | 3.24 | -1.02 |
| LOXL1 | 7.89 | 8.66 | 17.27 | 8.75 | 13.45 | 11.22 | -1.19 | -1.28 |
| ARMCX2 | 5.06 | 15.64 | 3.41 | 12.54 | 7.11 | 17.87 | -1.07 | -1.32 |
| LXN | 3.72 | 3.38 | 3.25 | 4.65 | 4.92 | 4.34 | 1.15 | 1.05 |
| CDH3 | 6.50 | 3.10 | 4.92 | 2.29 | 14.93 | 2.20 | 1.23 | 1.10 |
| MMP7 | 3.42 | 9.99 | 2.83 | 4.26 | 3.03 | 5.95 | 1.15 | 1.22 |

Table 3. Real-time qPCR and microarray detection fold change values

on silenced genes, 6 differentially expressed genes (H19, LOXL1, ARMCX2, LXN, CDH3 and MMP7) were selected for real-time qPCR. The results of real-time qPCR show that the 6 genes above in three EBV positive gastric cancer cell lines treated with Aza had different degrees of expression upregulation. However, 5 genes (LOXL1, ARMCX2, LXN, CDH3 and MMP7) in EBV negative gastric cell line SGC7901 have undifferentiated expression before and after Aza treatment, which is consistent with the result of gene chip. Only the expression of H19 gene up-regulated after treating EBV negative gastric cell line SGC7901, which is different from the result of gene chip detection. The comparisons between real-time qPCR and fold change values of chip detection are summarized in Table 3.

Candidate tumor suppressor genes promoter methylation detection

The MSP results show that LOXL1 gene promoter was highly methylated in GT38 and PT cell lines. Methylation and non-methylation were simultaneously detected in most cell lines, but methylation was not found in SGC7901 and HGC-27 cell lines. The results are exhibited in Figure 1.



Figure 1. Electrophoresis results of LOXL1 gene prompter methylation

In the 23 cases of EBVaGC tissue, two MSP types were detected in LOXLI coding gene promoter, including 12 cases of type M (12/23, 54.5%) and 11 cases of type M+U (11/23, 45.5%). Two MSP types were detected in the 23 cases of EBVaGC pericarcinomatous tissue, including 1 case of type M+U (1/23, 4.3%) and 22 cases of type U (22/23, 95.7%). In the 22 cases of EBVnGC tissue, two MSP types were detected in LOXLI coding gene promoter, including 15 cases of type M+U (15/22, 68.2%) and 5 cases of type U (7/22, 31.8%). Two MSP types were detected in the 22 cases of EBVnGC tissue, including 2 case of type M+U (2/22, 9.1%) and 20 cases of type U (20/22, 90.9%). The MSP detections of LOXLI coding gene promoter in EBVaGC and EBVnGC tissues are shown in Figure 2.



Figure 2. Electrophoresis results of EBVaGC and EBVnGCLOXL1 gene prompter methylation

BGS detected the methylation status of LOXL1 gene promoter of some samples. The sequencing results prove that in the 5 cases of EBV positive sample (GT38, PT, Akata, GC89 and GC95) with type M MSP result, most sites at CpG site in LOXL1 promoter region were methylated. In the 4 cases of Ebv positive samples (C666, GC98,

| Table 4. | LOXL1 | gene methylation | i rates |
|----------|-------|------------------|---------|
|----------|-------|------------------|---------|

| Group | Positive (case) | Negative (case) | Methylation rate (χ%) |
|--------------------------------------|-----------------|-----------------|-----------------------|
| EBVaGC (n=23) | 23 | 0 | 100% |
| EBVaGC matched para-carcinoma (n=23) | 1 | 22 | 4.3% |
| EBVnGC (n=23) | 15 | 7 | 68.2% |
| EBVnGC matched para-carcinoma (n=23) | 2 | 20 | 9.1% |

EBVaGC vs. *EBVaGC* matched para-carcinoma, $\chi^2=42.17$, P<0.01; *EBVnGC* vs. *EBVnGC* matched para-carcinoma, $\chi^2=16.20$, P<0.01; *EBVaGC* vs. *EBVnGC*, $\chi^2=6.41$, P<0.05.

Table 5. Relationship between LOXL1 gene methylation and the clinical pathological characteristics of gastric cancer patients

| Item | | Case No. | Positive | Negative | Positive rate (%) | χ^2 | P value |
|----------------------|---|----------|----------|----------|-------------------|----------|---------|
| Condor | Male | 39 | 32 | 7 | 82.1 | 1 20 | >0.05 |
| Gender | Female | 6 | 6 | 0 | 100 | 1.20 | -0.05 |
| 1 | <50 | 11 | 10 | 1 | 90.9 | 0.46 | >0.05 |
| Age | ≥50 | 34 | 28 | 6 | 82.4 | 0.40 | ~0.05 |
| Pathological type | Highly and mediumly differentiated adenocarcinoma | 9 | 8 | 1 | 88.9 | 0.17 | >0.05 |
| | Lowly differentiated adenocarcinoma | 36 | 30 | 6 | 83.3 | 0.17 | -0.03 |
| | Gastric cardia | 4 | 4 | 0 | 100 | | >0.05 |
| | Gastric body | 8 | 6 | 2 | 75.0 | | |
| Site | Gastric antrum | 20 | 16 | 4 | 80.0 | 2.37 | |
| | More than 2 sites | 10 | 9 | 1 | 90.0 | | |
| | Gastric stump | 3 | 3 | 0 | 100 | | |
| Lymphatic | Yes | 28 | 24 | 4 | 85.7 | 0.00 | >0.05 |
| metastasis | No | 17 | 14 | 3 | 82.4 | 0.09 | ~0.05 |
| EBV | EBVaGC | 23 | 23 | 0 | 100 | 6 11 | <0.05 |
| infection* | EBVnGC | 22 | 15 | 7 | 68.2 | 0.41 | ~0.03 |

**EBVaGC and EBVnGC*, $\chi^2 = 6.41$, *P*<0.05.

GC100 and GC81) with type M+U MSP result, CpG site in LOXL1 promoter region was partially methylated. In the 2 cases of samples (SGC7901 and HGC27) with type U MSP result, methylation did not occur at CpG site in LOXL1 promoter region. Moreover, in the 3 cases of EBVaGC tissue (GC98, GC100 and GC81), some CPG sites of LOXL1 coding gene had two different methylated forms, i.e. methylation (C) and non-methylation (T) existed simultaneously.

In EBV related and negative gastric cancer tissues, the positive methylation rate of LOXL1 gene promoter in ENV negative gastric cancer tissue (68.2%, 15/22) was significantly higher than that of pericarcinomatous tissue. The positive rate (100%, 23/23) of gastric cancer methylation was significantly higher than that of EBV (χ^2 =6.41 (correction), P<0.05). The results are shown in Table 4.

Relationship between LOXL1 gene methylation and clinical pathological characteristics

LOXL1 gene methylation in gastric cancer tissue and the gender, age, pathological histology type, diseased site, lymphatic metastasis and EBV infection of patients were compared (Table 5). The result show that LOXL1 gene methylation was not significantly correlated with the gender, age, pathological histology type, diseased site and morbidity of lymphatic metastasis. Only the methylation rates differed significantly (χ^2 =6.41(correction), *P*<0.05).

Discussion

EBV infection is closely associated with the occurrence of some gastric cancers. The results of in-situ hybridization showed that EBV could be de-

tected in almost all tumor cells in EBVaGC tissues, indicating that EBV infection occurred in the early gastric carcinogenesis and tumor originated from the monoclonal growth of EBV infected cells [11]. Further studies have shown that compared with EB-VnGC, EBVaGC has unique clinical pathological features. For example, the onset of EBVaGc presents a low-aging tendency, and the diseased part is commonly in the gastric body or cardia region at gastric fundus. The pathological type is mainly tubular adenocarcinoma or poorly differentiated adenocarcinoma. In terms of histopathological type, diffuse gastric cancer occurs more frequently than intestinal gastric cancer with low lymphatic metastasis rate and desirable prognosis [12]. The expression of virus coding gene of EBVaGc is not identical to that of EBV-related tumors such as NPC and Burkitt's lymphoma. For instance, recognized EBV cell transformation gene, i.e. latent membrane protein (LMP) 1 coding gene, is not expressed in EbVaGC [13]. Its differences from EBVnGC and other EBV-related tumors suggest that EBVaGC has a unique formation mechanism [14].

DNA methylation is the most cutting-edge epigenetic mechanism that has been extensively accessed, in which the role of methylationbased epigenetic change in tumorigenesis is of the greatest concern. In addition to mutation and deletion, the hypermethylation of tumor suppressor gene promoter is considered to determine the functional inactivation of tumor suppressor gene, which plays an important role in tumorigenesis and tumor evolution [15]. Currently, studies on EBVaGC methylation have been focusing on the detection of methylation status of known genes at DNA level, while methylation silenced cancer suppressor genes caused by EBV infection are rarely screened and identified. [16]. DNA methylation is not involved in the change of DNA sequence, therefore this change is reversible, suggesting that the hypermethylation status in gene promoter region can be eliminated by demethylation to reactivate silent genes, especially tumor suppressor ones [17]. Nucleoid methyltransferase inhibitors represented by 5-Aza-CdR have been widely used to reverse the aberrant methylation of tumor cells, induce the re-expression of methylation silenced tumor suppressor genes and inhibit the growth of tumor cells, thus killing tumor cells

[18]. EBV positive and negative gastric cancer cell lines were selected in this study as the object and treated with 5-Aza-CdR to re-express methylation silenced tumor suppressor genes. In the meantime, an untreated control group was established, the total cellular RNA of the experimental and control groups was prepared, and then the gene expression level was detected by high-throughput whole genome microarray expression profile chip to screen the silent cancer suppressor genes due to methylation. Then the methylation of these genes was verified and analyzed to identify methylation silenced cancer suppressor genes specific to EBV positive gastric cancer cell lines. Hence, the methylation status of target genes in EBVaGC and EBVnGC tissues was further detected and compared to analyze the relationship between the methylation of identified cancer suppressor genes and clinical pathological features so as to clarify the action mechanism of EBV infection in EBV positive cell lines and EBVaGC tissue methylating candidate cancer suppressor genes, and to define EBVaGC specific apparent molecular genetic marker.

Agilent gene expression profile [19] experiment: a comparative analysis was conducted on the differences of gene expression between 5-Aza-CdR treatment group and non-treatment group of EBV negative and positive gastric cancer cell lines. According to the different degrees of gene expression and SAS system and functional clues provided by relevant literatures, 6 differentially expressed genes (H19, LOXL1, ARMCX2, LXN, CDH3 and MMP7) were selected for real-time fluorescence quantitative PCR analysis to verify the results of gene chip [20]. The results show that 6 genes in three EBV positive gastric cancer cell lines with Aza treatment had different degrees of expression upregulation. Nevertheless, 5 genes (LOXL1, ARMCX2, LXN, CDH3 and MMP7) in EBV negative gastric cell line SGC7901 had undifferentiated expressions before and after Aza treatment, which is consistent with the result of gene chip. Only H19 gene had expression upregulation in EBV negative gastric cell line SGC7901 after treatment, which was different from the result of gene chip detection. The results above show that real-time fluorescence quantitative PCR accords with chip detection result, proving the reliability of gene chip result to some extent and indicating

that hypermethylation of these genes is caused by EBV infection, i.e. silent genes involved by EBV may be related to the occurrence of EBV-related gastric cancers [21].

This study found that LOXL1 gene promoter was hypermethylated in EBV-related gastric cancer tissues, indicating that DNA methylation status in LOXL1 gene regulation region is an essential regulatory mechanism of gene expression [22]. The MSP results show that LOXL-1 promoter was fully methylated in EBV positive gastric cancer cell lines GT38 and PT, partially methylated in EBV positive cell lines (B95-8, Raji, GT39 and SNU719), and unmethylated in EBV negative gastric cancer cell lines SGC7901 and HGC27. The methylation rate of LOXL-1 promoter in EBV-related gastric cancer tissues was significantly higher than that in EBV negative gastric cancer tissues. The results above reveal that the LOXL-1 gene methylation in EBVaGC tissues was highly correlated to EBV infection, and LOXL-1 gene was the candidate methylation silenced cancer suppressor gene of EBVaGC [23].

DNMT introduces an activated methyl group into DNA chain with SAM as the donor and forms m5C through covalent binding with carbon potential of genome CpG binuclear general acid [24]. Methylation transferases include maintenance methylation transferase (DNMTI) and remethylase (DNMT3a, DNMT3b). In the form of maintenance extensive methylation, these three enzymes have both overlapped and respective unique functions [25]. EBV is latently infected in tumor tissues. In EB-related tumor tissues such as Burkitt lymphoma and NPC, EBV genome is hypermethylated and close to the expression of nuclear antigen (EBNA) 2~6 genes and rise phase genes through methylation of Wp and Cp promoter genes and rise phase gene promoters Zp and Rp, thus avoiding the killing effect of cytotoxicity T cell on EBV infected cells [26]. Some studies also hold that virus and host cells interact in EBV carcinogenesis [27]. EBV can induce host cell gene to aberrant methylation while developing self-defense via its own gene methylation, which results in cell cycle disorder and cell transformation, as well as promotes the occurrence and development of EBV-related tumors epigenetically. This study shows that EBVaGc methylation frequency and methylation density of promoter CpG island were much higher than those of EBVnGC, and hypermethylation was negatively correlated with the expression of cancer suppressor genes in EBVaGc tissues such as p16 and p73. EBV infection induced aberrant methylation in CpG island, thereby inactivating cancer suppressor genes. It might be a mechanism of EBV being involved in the occurrence of EBVaGC. However, it is still undefined how EBV induces the methylation of host cell cancer suppressor gene. LMP1 can interact with cell cycle regulatory gene through multiple mechanisms and thus promote cell proliferation and suppress cell apoptosis. LMP2 and LMP1 are commonly distributed on cell membrane with synergistic effects, but the specific function remains unclear yet. LMP2A might be related to transmembrane signaling. In EBV-infected B cells, LMP2A can block the Ca²⁺ passage of B cells and suppress virus entering the lytic cycle, thus maintaining the latency infection of EBV. However, the function in epithelial cells has not been determined [28].

The study above indicates that the expression of virus-associated genes up-regulated that of DN-MTs by relevant signaling pathways, and finally induced aberrant methylation in CpG island, thus resulting in the inactivation of cancer suppressor genes. In EBV positive cell line and EBVaGC tissue, LOXL1 methylation degree was significantly higher than those of EBV negative cell line and EBVnGC tissue, inferring that EBV accounts for the DNA methylation in LOXL1 gene regulatory region and that LOXL1 gene might be the candidate methylation silenced cancer suppressor gene specific to EBVaGC.

In this study, EBV positive (PT, GT38 and SNU719) and negative (SGC7901) gastric cancer cell lines cultivated in vitro were treated with a demethylation reagent 5-Aza-CdR to extract the total cellular RNA before and after treatment. The results of gene chip were verified by real-time fluorescence quantitative PCR, and CpG island methylation in some gene promoter regions was detected by methylation specificity PCR (MSP) and hydrosulphite genome sequencing method (BGS). The gene (LOXL1) with reported tumor suppressor function was selected as the candidate cancer suppressor gene. In EBV positive cell line and EBVaGC tissue, LOXL1 gene promoter

region was hypermethylated and its methylation degree was significantly higher than those of EBV negative cell lines and EBVnGC tissue. Promoter CpG island had aberrant methylation and thus inactivated cancer suppressor gene [29].

In conclusion, the hypermethylation of cancer suppressor gene promoter governed the functional inactivation of cancer suppressor gene in addition to mutation and deficiency. EBV infection induced aberrant methylation of CpG island, which thus inactivated cancer suppressor gene. It might be a mechanism of EBV being involved in the occurrence of EBVaGC.

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Assessment of the role of progesterone in protecting against spinal cord injury via hypoxia-inducible factor-1alpha: An animal model study

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Abstract

Background: A spinal cord ischemia and neural hypoxia models were used to evaluate the neuroprotective effects of progesterone via hypoxia-inducible factor- 1α . We aimed to investigate the neuroprotective role of progesterone in spinal cord injury.

Methods: Eighteenfemale New Zealand white rabbits were randomized into three groups. Group 1(control) animals received nothing following reperfusion. Group 2 and 3 (treatment) animals received intraperitoneal progesterone immediately after the onset of reperfusion at a dose of 8 mg/kg. Spinal cords were fixed with 10% formalin and embedded in paraffin wax. The number of intact large motor neuron cells in the ventral grey matter region was counted. Cells expressing HIF1 were counted in high-power microscopic fields (x 400) in spinal cord sections around the areas of developing white matter necrosis.

Results: The number of intact neurons in group 1 was significantly lower than that in both groups 2 and 3 (p<0.05).HIF-1 α positivity was significantly higher in ischemic animals. HIF-1 α positivity in the non-ischemic arm of group 1 was significantly lower than that in the non-ischemic arm of groups2 and 3.

Conclusions: These data suggest that HIF-1 α plays an important role in hypoxic-ischemic preconditioning. Neuroprotective effects of progesterone may be mediated via the HIF-1 α .

Key Words: HIF-1 α , spinal cord injury, ischemia, progesterone.

Introduction

Acute spinal cord injury (SCI) is characterized by a progressive course which cannot be entirely explained by its primary mechanical trauma. A series of secondary injuries including ischemia, vascular changes, electrolyte disorders, edema and loss of energy metabolism, which can significantly increase the severity of SCI, have been observed in injured and adjacent segments after the acute post-injury phase [1]. Among all, secondary injuries, ischemia has been demonstrated as the main cause of post-injury pathophysiological changes of acute SCI since it has been believed to aggravate other secondary injuries and arises in parallel with neurological dysfunction.

Currently available treatment options for SCI are limited [2], and the standart drug therapy is aspirin [3]. Diverse therapeutic approaches (including prostaglandins, nimodipine, naloxone, adenosine, magnesium among others) have been used to control the damaging processes that can injure the spinal cord, eventually leading to its repair [4]. Among the limited number of treatments for SCI, currently available, are surgical decompression and the use of methylprednisolone, but these are considered to be ineffective [5]. Vascular endothelial growth factor (VEGF) is a unique neurotrophic factor. As a potential stimulator of angiogenesis, VEGF can improve locomotor function under hypoxic conditions following SCI [6].

It is now well documented that steroid hormones provide neuroprotection after injury of the central nervous system [7]. The list ofneuroprotective steroids has increased in recent years, and includes progestagens [8], androgens [9], and estrogens [10], which have been shown to decrease the extent of brain injury and to promote neuronal survival. The neuroprotective effects of progesterone after spinal trauma have also been demonstrated in some studies [11, 12].

Many studies have also investigated the mechanisms underlying hypoxic-ischemic brain damage, such as free radical formation [13], excitotoxicity [14], and inflammation [15,16]. Progesterone also reduces edema, necrosis, apopitosis, blood-brain barrier compromise, and the mediators of inflammation [17].

Hypoxia leads to an almost immediate shut down of general protein translation to decrease energy consumption during hypoxic energy starvation [18]. The protective effects of hypoxia may be regulated by improving tissue oxygenation via HIF- 1α and the up-regulation of its target genes. HIF- 1α is one of the best characterized stimuli for the induction of angiogenic response and of the expression of several genes in a variety of tissues including vascular endothelial growth factor (VEGF) [19,20]. In addition to VEGF, HIF-1a activates genes encoding erythropoietin (EPO), glucose transporters and glycolytic enzymes, cell survival factors, cell surface receptors, extracellular matrix proteins and transcription factors [19,20]. Hypoxia-inducible factor-1 α (HIF-1 α), which was first identified in 1988 in human hepatoma cells as a key factor mediating the transcription of target genes [21], has been intensively investigated for its role in the modulation of hypoxic-ischemic brain injury since 1995 [22,23]. In our present study, we aimed to investigate whether progesterone demonstrates a neuroprotective effectiva HIF-1 α in rabbits.

Materials and Methods

Animals

A total of 18 female New Zealand white rabbits (8-12 months old), each weighing between 2.4 and 3.5 kg, were used in this study. All animals were housed under standard conditions in the Animal Research Laboratory at DokuzEylul University. The study protocol was approved by the animal research committee. The animals were fasted for

12 hours and humanely restrained. Anesthesia was induced with 3% halothane in 100% oxygen and was maintained with 0.5% to 1.5% halothane in a mixture of 50% oxygen and 50% room air. End-tidal concentrations of halothane and CO_2 were-continuously measured with monitor (Anesthetic Gas Monitor Type 1304; Brüel&Kjaer, Naerum, Denmark) via nasopharyngeal sampling.

The retroauricular vein in the right ear was cannulated, and an infusion of 0.9% NaCl solution was started at a rate of 4 mL/kg per hour. An artery in the left ear was also cannulated to monitor arterial blood pressure and allow for arterial blood gas sampling. To monitor proximal and distal aortic pressures, catheters were placed into the aorta and the femoral arteries. Verification that the appropriate level of sedation had been reached was determined by the lack of a righting reflex and by testing the palpebral and pedal withdrawal reflexes every 10 minutes, as previously described by Wyatt et al. [24]. Allexperiments were performed under the same conditions. Rectal temperatures were maintained at 38.5°C by keeping the animals under a heat lamp until their recovery from anesthesia.

Surgical procedures

The sedated animals continued to breathe spontaneously and were placed in the right lateral decubitus position. The skin was prepared with povidone iodine and anesthetized with bupivacaine (25% solution), and an incision was made in the flank, parallel to the spine at the 12th intercostal level. Following incision and dissection throught the thoracolumbar fascia, the longissimuslumborum, and iliocostalislumborum muscles were retracted. The abdominal aorta was exposed via a left retroperitoneal approach and mobilized just inferior to the left renal artery, where it was clamped, down to the point of the aortic bifurcation. Each rabbit was anticoagulated with 400 U of heparin before aortic occlusion. After 30 minutes of occlusion, the catheters were removed, and the incision was closed. The animals were monitored until they fully recovered and were then returned to their cages.

Experimental design

The animals were randomly divided into three groups, each consisting of six rabbits. Group 1(control) animals received nothing following reperfusi-

on. Group 2and 3 (treatment) received intraperitoneal progesterone (Progynex 50mg/m) immediately after the onset of reperfusion at a dose of 8 mg/kg. This specific dose of progesterone was chosen because it has been shown in multiple studies to prevent neuronal loss after brain injury and ischemia [25,26]. After completion of the surgical procedures, the tube and catheters were removed, and the incision was closed. Two hours after reperfusion, the animals in group 1 were killed. Four and six hours after reperfusion, the animals in groups 2 and 3, respectively, were killed with intraperitoneal sodium thiopental (120 mg/kg). The spinal cords from all animals were removed and fixed in 10% formalin in a phosphate buffer.

Determination of the physiologic parameters and progesterone levels

During the surgical procedure, the heart rate, mean arterial pressure, and rectal temperature were continuously monitored (Biopac MP30 and Biopac BSL pro v.3.6.5; Biopac Systems, Santa Barbara, CA), in addition to the respiration rate and end-tidal CO₂ level. Following surgery, the rabbits were placed in a warming chamber, and their body temperatures were maintained at approximately 37°C until they were completely awake. Blood samples (2 mL) were taken from the peripheral veins of all the animals before surgery and before sacrifice to measure the serum progesterone levels. Once the postsurgical progesterone levels were determined, the animals were killed. Blood samples remained at room temperature for one hour, until they clotted. Samples were then centrifuged for 10 minutes at 4000 rpm to obtain serum specimens. Serum specimens were stored at 4°C and analyzed within 24 hours to determine the progesterone level, which was measured according to the colorimetric method of Bar-Or et al [27].

Histopathology

Spinal cords were removed and fixed in 10% formalin in a phosphate buffer. After fixation, transverse sections of the spinal cord at the L5 level were embedded in paraffin, cut into 5-µm-thick sections, and stained with hematoxylin and eosin. Neuronal injury was evaluated at x40, x100, x200, and x400 magnifications by a pathologist who was blinded to the treatment groups. Five sections per

animal were read. We performed hematoxylin and eosin staining on a set of sections and examined them using light microscopy. The number of intact large motor neuron cells in the ventral gray matter region was counted. The observers, who were blindto the experimental groupings and neurologic outcomes, examined each slide. Following hematoxylin and eosin staining, the cells were considered to be dead if the cytoplasm was diffusely eosinophilic and were considered viable if the cells demonstrated basophilic stippling.

Immunohistochemistry

All specimens were fixed with 10% formalin and embedded in paraffin wax. Paraffin blocks were cut into 4µm sections and stained with hematoxylin and eosin. Immunohistochemical studies were performed on formalin fixed, paraffin-embedded tissue. The slides were stained on a DAKO Autostainer (DAKO Denmark) using the LSAB + System- HRP (Dako) staining reagents. The sections were then incubated with a monoclonal antibody specific for anti HIF-1 alpha (Bioss-USA) at a 1: 100 dilution of the original antibody solution for 60 minutes. Diaminobenzidine (DAB) was used as a chromogen for reaction visualization. Finally the sections were counterstained with Mayer's hematoxylin, dehydrated, cleared with xylene and mounted with coverslips using permanent mounting medium. Nuclear staining was used as a positive counterstain. Non papillary renal cell carcinoma was used as a positive control. Immunostained cell counts were made by two pathologistwho were blind to the subject data. Cells expressing HIF1 were counted in high-power microscopic fields (x 400) in spinal cord sections around areas of developing white matter necrosis.

Statistical analysis

For statistical evaluations, we used the software package SPSS for Windows v.15.0 (SPSS, Inc, Chicago, IL). Data from all groups are expressed as the mean \pm SD. A probability value of less than 0.05 was accepted as statistically significant. Because the variances were not homogenous (Levene's test statistic p<.05), post hoc Dunnett's T3 analysis was performed to determine from which group any significant differences in the findings had arisen.We used non-parametric tests, because of the small size of groups. The Kruskal–Wallis one-way analysis of variance and the Mann-Whitney U test were used to evaluate values.

Results

The mean value sat baseline (before the surgical procedure), before clamping and before sacrifice (sac) as well as the mean number of non-ischemic neurons and mean progesterone levels for the three groups are listed in Table 1. Statistically significant differences were identified between the baseline mean arterial pressure (MAP) and sac-MAP values of the groups (p<0.05). This analysis revealed that the average baseline MAP value for group 1 was significantly higher than that for group 2, and the mean sac MAP value for group 1 was significantly higher than that for group 3 (p<0.05). The number of intact neurons in group 1 was significantly lower than the number of intact neurons found in both groups 2 and 3 (p<0.05). No other statistically significant differences were found between thegroups, in terms of their mean PROG heart rate (HR) and PROG saturation (SAT) values (p>0.05) [Table 2]. No statistically significant correlation was found between the number of live neurons in the ventral grey area and progesterone levels (either pre- or post-surgery) in any of the groups (p>0.05) [Table3].

Table 1. Distribution of mean base, clamp, SAC, intact neurons, base progesterone, and after progesterone values of cases among groups

| <i>U</i> | 00 1 | | | | |
|--------------|--------------------|--------------------|--------------------|------------------|----------------|
| | Group 1 Mean±SD | Group 2 Mean±SD | Group 3 Mean±SD | Total Mean±SD | <i>P</i> value |
| Base MAP | 98.5±7.74 | 79.83±8.82 | 88.5±9.77 | 88.94±11.41 | .008 |
| Clamp MAP | 92.67±14.67 | 82.17±5.12 | 80.83±6.43 | 85.22±10.62 | .101 |
| SAC MAP | 82.5±17.18 | 74.33±18.79 | 55.67±14.08 | 70.83±19.56 | .040 |
| Base HR | 288.17±7.6 | 265.67±22.92 | 262.83±20.96 | 272.22±20.9 | .061 |
| Clamp HR | 239.67±15.62 | 248.67±36.52 | 264±17.3 | 250.78±25.67 | .265 |
| SAC HR | 238±14.14 | 240.5±29.04 | 275.67±34.09 | 251.39±31.01 | .052 |
| Base SAT | 99.17±1.6 | 99.83±0.41 | 99.67±0.52 | 99.56±0.98 | .774 |
| Clamp SAT | 99±1.67 | 99.17±1.17 | 99.5±0.55 | 99.22±1.17 | .438 |
| SAC SAT | 99.17±0.75 | 99.17±1.17 | 99.33±0.82 | 99.22±0.88 | .001 |
| Intactneuron | 23.17±4.49 | 37.17±6.91 | 34.5±4.59 | 31.61±8.07 | .217 |
| Base PROG | 0.72±0.59 | 8.23±14.58 | 0.24±0.27 | 3.06±8.77 | .217 |
| After PROG | 0.74±0.59 | 98.17±142.95 | 117.51±179.58 | 72.14±135.13 | .292 |

Values are the mean \pm *SD*; *n*=6 *in each group. Sat, saturation; PROG, progesterone levels; clamp, clamping time.*

Table 2. Distribution of mean progesterone values of the cases in groups 2 and 3

| | Group 2 Mean ±SD | Group 3 Mean ±SD | Total Mean ±SD | P value |
|----------|---------------------|---------------------|-------------------|---------|
| PROG MAP | 54.83±11.86 | 74.17±14.7 | 64.5±16.25 | .031 |
| PROG HR | 242.33±35.38 | 269±22.15 | 255.67±31.4 | .149 |
| PROG SAT | 99.5±0.84 | 99±1.26 | 99.25±1.06 | .938 |

Values are the mean \pm *SD*; *n*=6 *in each group. PROG, progesterone treatment time.*

Table 3. Correlation between number of intact neurons and base progesterone and after progesterone values of the cases in groups 1, 2 and 3

| | Group 1 | | Group 2 | | Group 3 | |
|------------|---------|-------|---------|-------|---------|------|
| | R | Р | R | Р | r | Р |
| Base PROG | 0.294 | 0.572 | -0.062 | 0.907 | -0.556 | .252 |
| After PROG | 0.309 | 0.552 | -0.348 | 0.499 | 0.472 | .344 |

Base PROG, progesterone levels in the blood before progesterone treatment. After PROG, progesterone levels in the blood after progesterone treatment.

| | | Group 1 (n=6) | Group 2 (n=6) | Group 3 (n=6) | <i>P</i> value ^b | |
|-----------------------|-----------------------------|------------------|------------------|------------------|-----------------------------|--|
| | | 0 | 0 | 0 | | |
| | N | 0 | 1.5 | 0.5 | | |
| | Nonischemic | 0 | 1 | 0 | 07 | |
| | (n=6) | 0 | 1.2 | 0 | .02 | |
| | | 0 | 1 | 0.5 | | |
| | | 1 | 1 | 0 | | |
| HIF-1 Positivity | Ischemic Group (n=6) | 3 | 3 | 1.2 | | |
| 1 Usitivity | | 4 | 2 | 2 | 00 | |
| | | 3 | 1 | 1 | | |
| | | 3 | 1.5 | 1.3 | .08 | |
| | | 1.5 | 2.2 | 1.3 | | |
| | | 1 | 1.5 | 1.3 | | |
| | <i>P</i> value ^a | .003 | .027 | .003 | | |

Table 4. Comparative analysis of HIF-1a positivity among group

^{*a*}:The difference between ischemic and nonischemic subjects in each group.

^b:The difference between the groups according to the state of ischemia.

It was limited to evaluate the number of intact large motor neuron cells by light microscopy. Thus, we explored in this study the effectiveness of progesterone as a treatment for SCI. We investigated the neuroprotective effects of progesterone by evaluating HIF-1 α positivity. In the experiments, HIF-1 α positivity was significantly higher in ischemic groups than without ischemic groups. In addition, HIF-1 α positivity in the non-ischemic arm of group 1 was significantly lower than in the non-ischemic arm of group 2 and 3. There was no significant difference in the HIF-1- α positivity among the ischemic arms of the patient groups (Table 4).

Discussion

Traumatic SCI causes devastating neurological dysfunction primarily via necrotic damage and following secondary injury events including ischemia, excitotoxicity, altered ionic balance, free radical formation, and inflammatory responses [28].To date, much effort has been expended to elucidate the molecular mechanisms within neurons that mediate neuronal death during ischemia and hypoxia. Studies that will further elucidate the pathophysiological background of ischemia are thus needed to identify novel therapeutical strategies.

A number of potential alternative therapies for SCI have been proposed and tested but they have failed to yield effective improvements.Some studies have reported positive results with certain drugs (including prostaglandins, nimodipine, naloxone, adenosine, and magnesium) [11]. Furthermore, there have been some studies of the efficacy of antihrombotic treatment for SCI [29,30]. Corticosteroid is also one of the alternative therapeutic approaches [31]. In addition, some studies have also reported potential benefits of progesterones [11, 32], androgens [9], and estrogens [10].

Once a destructive process is initiated such as SCI, the release of pro-inflammatory cytokines further stimulates immune cells to become phagocytic. Progesterone decreases the mediators of inflammation [33,34]. In some studies, it was reported that natural progesterone given to both males and females may easily cross the blood-brain barrier and dramatically reduce edema to barely measurable levels in an injured animal brain [35,36]. Progesterone may also reduce lipid peroxidation and the generation of isoprostanes, which in turn contribute to postinjury hypoxic-ischemic conditions [37]. In this context, it will be important to investigate the relationship between hypoxic exposure and therapeutic agents such as progesterone in future studies.

HIF-1 α may act as a critical regulatory factor for those of its target genes associated with the modulation of glycolysis and re-establishment of microcirculation in SCI. HIF-1 α isalso involved in ischemia [38]. Kalesnykas et al. have reported that HIF-1 α increased in rat neurons after unilat-

eral occlusion of a common carotid artery [39]. The authors of that study suggest that decreased blood flow and ischemia resulted in cellular hypoxia during the common carotid artery occlusion, leading to stabilization HIF-1 α . Other studies have shown that HIF-1 α protein levels increase immediately after the hypoxic exposure, peak at 3-4 hours after hypoxicischemic injury, and persist at elevated levels for up to 24 hours after the insult [22]. As mentioned earlier, hypoxic ischemia is an important cause of spinal injury. However, a sublethalhypoxic/ischemic exposure can improve the tolerance of tissue or of cells to a subsequent lethal hypoxic/ischemic insult. This phenomenon is called hypoxic/ischemic preconditioning (H/IPC) [40]. Some studies support the hypothesis that HIF-1 α plays an important role in H/ IPC, and that the protective effects of H/IPC may be partially mediated by improving tissue oxygenation via HIF-1 α and upregulation of its target genes. In our present study, we aimed to prove that progesterone has neuroprotective effects via HIF-1 α .

Our current results are consistent with reported findings in the literature. HIF-1 α positivity has been reported previously to be significantly higher in ischemic groups than non- ischemic groups [22, 38]. Thus, HIF-1 α possibly participates in the ischemic and hypoxic pathways that operate after SCI, and may mediate the traumatic process involved.In our current study, HIF-1 α positivity in the non-ischemic arm of group 1 was found to be significantly lower than in the non-ischemic arm of groups 2 and 3.We thus concluded that progesterone increases HIF-1 α and induces neuronal improvement. This result is consistent with other studyfindings in terms of neuroprotective effects [11]. Ultimately, our present data support he neuroprotective effects of progesteroneagainst SCI.

Limitations

The rapid acceptance of immunohistology as an invaluable adjunct to morphologic diagnosis has been possible because of the development of new and more sensitive antibodies and detection systems that allow its application to formalin-fixed, paraffin-embedded tissue (FFPT. While it was not a major issue when the technique was employed in a qualitative manner, the numerous variables in the preanalytical and analytical phases of the test procedure that influence the immunoexpression of proteins in FFPT become critical to standardization. Tissue fixation is pivotal to antigen preservation but exposure to fixative prior to accessioning by the laboratory is not controlled. There is great variation in reagents, methodology, and duration of tissue processing and immunostaining procedure, and the detection systems employed are not standardized between laboratories. While many of these variables are offset by the application of antigen retrieval, which enables the detection of a wide range of antigens in FFPT, the method itself is not standardized. Failure to recognize false-positive and false-negative stains leads to further errors of quantitative measurement.

Conclusion

Our present data suggest that progesterone administration facilitates neuronal protection through a hypoxic inducible system in SCI. The involvement of HIF-1 α after spinal injury brings new insights into the role of progesterone in neuroprotection.

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The general reconstruction plate combination with cancellous screws for the management of unicondylar hoffa fractures

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Abstract

Objectives: Isolated coronal fractures of the femoral condyle (Hoffa fractures) are uncommon injuries. The current diagnosis and treatment of these fractures is mainly based on a few descriptions of sporadic cases in literature. We reported 7 Hoffa fractures treated at a Level I trauma center by one surgeon and systematically analyse the injury mechanism, diagnostic method, surgical technique, radiological and clinical outcome of Hoffa fractures.

Patients and methods: We reviewed all case records of 7 unicondylar Hoffa fractures treated by one surgeon from May 2007 to Jun 2011. All the patients were isolated unicondylar Hoffa fracture. Of 7 such cases, 5 were of lateral condyle and 2 were medial femoral condyle fractures. Each patient suffered a high-energy injury including a patient of delayed presentation for two months. There were 4 patients suffering road traffic accident, one patient suffering fall from height and 2 patients suffering bruise injury by weight. All fractures were treated by open reduction through a typical posterolateral approach or posteromedial approach described by Henderson. Internal fixation was performed with general reconstruction plate combination with cancellous screws for all cases. Postoperatively, The affected knee began immediate unrestricted range of mation. The limited weight bearing started at the fourth week after surgery, and all patients were permitted full weight bearing at eighth week. Reduction quality and radiological follow-up results were assessed in standard anteroposterior and lateral radiographs. Clinical outcome results were evaluated by Knee Society scores after average 34 months follow-up time.

Results: All fractures healed at a mean period of 4.0 months. Long-term follow-up (average 34 months, range 24-50 months) was available for all

patients. All fractures achieved anatomical reduction but one patient who was delayed presentation for two months achieved acceptable reduction. There was no loss of reduction or failure of fixation. Early complication was encountered in one case with wound infection but healed within 2 weeks owing to the help of efficient antibiotics. Late complication was found in one case with stiffness and pain of the affected knee. The final clinical outcome was excellent in 4 patients, good in 2 patients and poor in 1 patient.

Conclusion: Isolated Hoffa fractures are rare intraarticular injury and there is a highly likelihood of these fractures being missed by an orthopaedic surgeon. Oblique radiographs and CT scans can help to confirm and define these fractures, especially in cases of undisplaced fractures. Typical posterolateral/posteromedial approach described in our study can provide the direct visualization of the fracture and articular surface. Internal fixation using a common reconstruction plate combination with cancellous screws can obtain a stable and rigid fixation and lead to a good functional outcome in the long term.

Key words: Hoffa fracture, Coronal fracture, Unicondylar, Reconstruction plate, Cancellous screws.

Introduction

Coronal fractures of the femoral condyle are uncommon injuries and were first described by Hoffa in 1904¹. They are classified as a Type 33-B3 fracture by the Orthopaedic Trauma Association². Isolated Hoffa fractures are rare injuries, but they often occur with intercondylar and supracondylar fractures³. By definition, these fractures are intra-articular injuries, and hence, the principles of management demand anatomical reduction and internal fixation to enable early mobilisation and to prevent arthrofibrosis⁴. Although preliminary methods for the diagnosis and management of these fractures have been published in sporadic case reports, very few studies have systematically analysed the injury mechanism, diagnostic methods, surgical techniques and long-term radiological and clinical outcomes of Hoffa fractures. Therefore, we performed a retrospective study to evaluate the results of the surgical management of isolated Hoffa fractures.

Materials and methods

We retrospectively analysed 102 cases involving femoral condyle fractures operated on by one surgeon from May 2007 to Jun 2011. Seventeen patients with Hoffa fractures were selected. Three patients with related supracondylar or inter-condylar fracture components were excluded. Two patients with ipsilateral proximal femur injuries, two patients with bilateral Hoffa fractures, one patient with pathological fractures and one patient with pre-existing severe osteoarthritis were also excluded. After exclusion, 7 patients with isolated unilateral Hoffa fractures were enrolled in the study, including 6 males and 1 female. The average patient age was approximately 40 years old (range 28-59 years). The preoperative assessment included a routine laboratory examination, a clinical examination (such as the swelling degree of the affected knee, the condition of the heart and lung function) and imaging examinations, including anteroposterior (AP) and lateral X-rays of the affected knee. A three-dimensional computed tomography (CT) scan was taken for each case to assess the fragment size and make a surgical plan. MRI was performed if there was a high index of suspicion of meniscal, cruciate ligament or lateral collateral ligament injury. Hoffa fractures were classified into three sub-types according to Letenneur et al⁵ (figure 1). There were two type 1 fractures, two type 2 fractures and three type 3 fractures in our study. All of the fractures were obviously displaced. Five fractures involved the lateral condyle, and two fractures involved the medial condyle. Five patients injured their left knee, and two patients injured their right knee. No patient suffered subluxation or dislocation of the knee joint. All fractures were closed injuries, and there were

no associated neurovascular injuries. Each case had suffered a high energy injury, including traffic accident in four cases(motor vehicle accidents in 3 cases, a car accident in 1 case), a fall from a height in 1 case and bruising injury by weight in 2 cases. The mean time from injury to surgery was 5 days (4-7 days), except one patient who delayed presentation for 2 months before admission. This patient had a bruising injury by weight due a housing collapse. Although the affected knee was swollen and tender upon examination, the local orthopaedic surgeon failed to recognise the medial Hoffa fracture in the anteroposterior (AP) and lateral X-rays. The injured knee was immobilised by a tubular cast in 20° flexion for nearly two months, and the extension and flexion function of ipsilateral knee were 10° and 40°.(Figure 2).



Figure 1. Letenneur classification of the Hoffa fractures



Figure 2. Preoperative anteroposterior and lateral radiographs and a CT scan of the patient with delayed presentation

Open reduction and internal fixation were adopted for all fractures. Preoperative antibiotics (cefazolin sodium 2000 mg) and general anaesthesia were administered to every patient. A tourniquet was placed around the ipsilateral thigh and inflated at the surgeon's discretion. We used a typical posterolateral approach for the lateral Hoffa fractures, while the posteromedial approach was

| No. | Age/Sex | Injury history | Injury side | Affected condyle | Subtype | Associated injury | Time to surgery |
|-----|-----------|------------------------|----------------|------------------|----------|-------------------|--------------------|
| 1 | 59/male | Bruise by weight | Left | Medial | Type III | Cruciate ligament | 4 days |
| 2 | 46/male | Bruise by weight | Left | Lateral | Type I | Meniscus | 6 days |
| 3 | 32/male | Motor vehicle accident | Right | Lateral | Type III | Meniscus | 4 days |
| 4 | 41/male | Car accident | Left | Lateral | Type II | Meniscus | 5 days |
| 5 | 28/male | Motor vehicle accident | Right | Lateral | Type III | Cruciate ligament | 5 days |
| 6 | 36/male | Fall from height | Left | Lateral | Type II | Meniscus | 7 days |
| 7 | 37/female | Motor vehicle accident | Left | Medial | Type I | NO | 4 days |

Table 1. General presentations of the patients

used for the medial Hoffa fractures. Both surgical approaches were described by Henderson⁶. All fractures were treated with a general reconstruction plate combination with cancellous screws. We recorded the operative time and intraoperative blood loss. Postoperatively, active range of motion exercises were started after removing the drainage tube from the wound, and a supervised physiotherapy programme was initiated at the Rehabilitation Department after discharge. Initially, the patients were restricted to non-weight bearing activities, but they progressed to full weight-bearing activities by 6 weeks. Patients were discharged at a mean of 11 days postoperatively (9-16 days). All of the patients were compulsory for follow-up visits at a clinic service at 4, 8 and 12 weeks and at 6 and 12 months. The results of the clinical examinations and imaging findings were recorded. Knee Society clinical scores (KSSs) were obtained at the final follow up (Table 1).

Surgical Technique

The patients were placed in a supine position on a radiolucent table. A typical posterolateral approach was used for lateral Hoffa fractures. With the knee flexed 90 degrees horizontally, we made a curved incision on the lateral side of the knee, just anterior to the biceps femoris tendon and the head of the fibula and avoided the common peroneal nerve, which passes over the lateral aspect of the neck of the fibula. In the proximal part of the incision, we traced the anterior surface of the lateral intermuscular septum to the linea aspera 7 cm proximal to the lateral femoral condyle, after exposing the lateral femoral condyle and the origin of the fibular collateral ligament. We could then see that the tendon of the popliteus muscle lies between the biceps tendon and the fibular collateral ligament. Subsequently, we mobilised and retracted it posteriorly and exposed the posterolateral aspect of the joint capsule. After that, a longitudinal incision was made through the capsule and synovium of the posterior compartment. The lateral distal femur, fracture site and lateral articular surface were then in full view. Exploration was performed carefully and thoroughly for each patient to judge if there was an associated injury, such as meniscus or cruciate ligament injury. Surgical intervention was performed if a related injury existed. Reduction and compression of the major fracture components were performed with a pointed reduction clamp placed posterior to anterior. Temporary fixation of any intercalary osteochondral fragments and the major condylar fragments was achieved with Kirschner wires. The reduction was confirmed with the help of fluoroscopic imaging of the C-arm. After the optimal reduction was obtained, the fracture site was fixed with two or more cancellous screws (3.5 mm diameter) placed posterior to anterior. The heads of the screws were buried into the cartilage. The screw directions were ideally perpendicular to the major coronal plane fracture and along the longest axis of the medial femoral condyle. Thereafter, the general reconstruction plate was pre-bent to an arc configuration that appropriately matched the physiological arcuation of the posterior condyle of the femur. To withstand the shearing force in a large extent and avoid the failure of internal fixation, we placed the general reconstruction plate on the posterior surface of the distal femur and confirmed that the inferior margin of the reconstruction plate was above the cartilage surface of the lateral condyle. After fixation, the accuracy of reduction and implant placement was confirmed by direct visualisation and radiographically.

For medial Hoffa fractures, the posteromedial approach was used. Analogously, the patient was placed in a supine position on a radiolucent table. With the knee flexed 90 degrees, we made a curved incision that was slightly convex anteriorly and approximately 12 cm long. The incision ran distally from the adductor tubercle and along the course of the tibial collateral ligament, anterior to the relaxed tendons of the semimembranosus, semitendinosus, sartorius, and gracilis muscles. We then exposed and incised the oblique part of the tibial collateral ligament. The capsule was longitudinally incised, and the posteromedial compartment of the knee was entered posterior to the tibial collateral ligament, with posterior retraction of the hamstring tendons. At this time, the lateral distal femur, fracture site and medial articular surface were in full view. After exploration, the reduction and fixation of the medial condyle fracture were started as described above in the treatment of lateral condyle fractures.

Results

All patients were followed up for a mean period of 34 months (range 24-50 months). The mean operative time was 104 min (80–150 min). The mean blood loss was 347 ml (250–500 ml). There were 4 patients with meniscus injuries and 2 patients with cruciate ligament injuries, which were all repaired intraoperatively. One patient suffered an early complication of superficial skin infection, but it was ultimately cured through the use of a second-generation cephalosporin for approximately one week. Articular reductions were classified as anatomical, acceptable (<2 mm step) and poor (>2 mm) on the immediate postoperative radiographs. Six of the 7 cases achieved anatomi-

cal reduction, while the case with delayed presentation had a <2 mm step off. AP and lateral X-rays of the affected knee were taken for each case during the follow-up visits to assess the progression of the union and to determine whether there was a loss of reduction or failure of fixation. Fracture healing was clinically defined as the ability to bear weight without pain and radiologically defined as the disappearance of the fracture line and evidence of bridging trabeculae across the fracture lines. Fracture union was obtained in all cases, and there was no loss of reduction or failure of fixation at the final follow-up visit. There was no evidence of osteonecrosis or the development of degenerative arthritis at the final follow-up visit. However, a late complication was encountered in one case with stiffness and pain of the affected knee. The range of knee motion achieved a good result in all but one patient. The mean KSS at the final followup visit was 78.7, and the evaluation results were excellent in 4 patients, good in 2 patients and poor in 1 patient (Table 2, Figure 3 and Figure 4).



Figure 3. The patient with delayed presentation was treated using a general reconstruction plate combination with cancellous screws

| No. | Surgical approach | Surgical time | Blood loss | Complications | Knee ROM | Length of stay | Knee Society score |
|-----|----------------------|------------------|---------------|-------------------|----------|-------------------|-----------------------|
| 1 | Posteromedial | 95 min | 280 ml | NO | 0~140° | 9 days | 86 |
| 2 | Posterolateral | 105 min | 360 ml | NO | 0~130° | 10 days | 85 |
| 3 | Posterolateral | 120 min | 420 ml | Wound infection | 0~120° | 16 days | 78 |
| 4 | Posterolateral | 85 min | 300 ml | NO | 0~135° | 11 days | 75 |
| 5 | Posterolateral | 90 min | 320 ml | NO | 0~125° | 10 days | 92 |
| 6 | Posterolateral | 150 min | 500 ml | Stiffness of knee | 10~90° | 11 days | 55 |
| 7 | Posteromedial | 80 min | 250 ml | NO | 0~130° | 10 days | 80 |

Table 2. The intraoperative and postoperative results of the patients



Figure 4. Anteroposterior and lateral radiographs of the patient with delayed presentation after two years of follow up.

Discussion

Hoffa fractures are isolated, coronally oriented fractures of a femoral condyle with intra-articular extension. The lateral condyle is more commonly affected than the medial condyle^{4-5,7}. The physiological valgus of the knee joint may explain the preponderance for fractures to occur more often in the lateral condyle. Many studies have shown that bicondylar injuries are very rare^{3,8-13}, while injuries associated with Hoffa fractures are common and often occur in the ipsilateral knee^{3,8}, lower extremities¹⁴⁻¹⁵, or more distant locations^{3,7,16}.

These rare injuries belong to Salter and Harris type 4 and OTA type 33-B3 fractures (frontal, partial articular fractures of the distal femur). These injuries were previously classified by Letenneur⁵ et al based on the distance between the fracture line and the posterior cortex of the femoral shaft. The original intention of classification was an attempt to predict which coronal plane fractures of the femoral condyle would progress to avascular necrosis. Type I fractures extend from an extra-articular location at the junction of the posterior femoral shaft and the proximal aspect of the femoral condyle superiorly to the posterior aspect of the condylar articular surface and inferiorly, such that the popliteal tendon insertion and the lateral head of the gastrocnemius origin remain attached to the condylar fragment. The anterior cruciate and lateral ligament insertions may be attached to either the condylar or shaft fragment. Type II fractures originate posterior to the posterior femoral shaft-condylar junction and are therefore potentially entirely intra-articular. Compared to type I fractures, the aforementioned ligamentous insertions are less likely to be attached to the condylar fragment. In type III fractures, all of the ligamentous insertions remain attached to the condylar fragment. However, there is currently no conclusive correlation between the incidence of avascular necrosis and the fracture type.

Hoffa fractures correlate significantly with high velocity, high energy trauma. However, the exact mechanism of injury is not certain. Some hakeems have postulated that a shearing force on the posterior femoral condyle is the mechanism of injury^{4,17}. This mechanism of injury would involve a force resulting from the impact of the upper part of the tibia on the femoral condyles, particularly the lateral condyle, with the knee flexed more than 90°. However, others have attributed this fracture type to simultaneous vertical shearing and twisting forces^{13,18}. Most of these injuries are caused by motor vehicle accidents, while a fall from a height appears to be the next most common cause^{3-4,17}. In our study, however, we found that bruising injury by weight was an infrequent cause of injury (two cases). In this type of injury, the associated forces of vertical shearing and twisting may be the primary mechanism of injury.

The diagnosis of a Hoffa fracture is challenging and is often missed by orthopaedists. It may be difficult to make an exact diagnosis from the initial anteroposterior and lateral radiographs because Hoffa fractures are hard to recognise, particularly when they are undisplaced ^{3-4,19-20}. On the anteroposterior view, the foreshortened fractured condyle may lead to the appearance of varus or valgus malalignment. On a standard lateral view, the two femoral condyles are not superimposed, but this may be misunderstood as a poor radiographic view. Oblique radiographs may assist in identifying minimally displaced fractures that are not visible on other views¹⁵. In cases with a high index of suspicion but in which no conclusions can be drawn, axial computed tomography with sagittal reformations is becoming the gold standard for diagnosis and characterisation of intraarticular fractures^{3-4,19-20}. In our study, one fracture was missed initially at the patient's local hospital because the local doctors did not recognise the Hoffa fracture from the simple anteroposterior and lateral radiographs.

The surgical indications for Hoffa fractures are poorly defined, but open reduction and internal fixation are necessary to achieve satisfactory results following a Hoffa fracture. Nonoperative management has been shown to result in further displacement, malunion and nonunion^{4-5,20-23}. Hoffa fractures, as articular fractures, require open reduction and stabilisation with good exposure of the fracture fragments posteriorly. However, the surgical approach to coronal fractures of the distal femur is controversial. Generally, the articular surface is exposed through a medial or lateral approach, depending on which condyle is involved. A midline incision with a lateral parapatellar arthrotomy for a lateral Hoffa fracture has been reported numerous times as the most common approach¹⁷. However, the direct lateral approach with or without osteotomy of the gerdy tubercle and posterior-based approaches have also been described. Advocators have argued that this approach can allow direct access to the articular aspect of the fracture^{4,25,27-28}. As for medial Hoffa fractures, an anterior midline incision with a medial parapatellar approach is popular and has favourable outcomes^{17,26-28}. Some scholars have argued that, although the parapatellar approach allows access for reduction anteriorly, the visualisation of posterior comminution and the ease of reduction may be limited²⁹. Meanwhile, they recommend a medial subvastus approach as a potential alternative for medial Hoffa fractures because it allows for extensile exposure of the articular surface and access to the posterior femoral condyle for the placement of fixation in multiple planes. Furthermore, the medial subvastus approach has been shown to have the potential advantage of preserving the extraosseous blood supply of the medial condyle and hence minimising the surgical vascular insult. The procedure can thereby potentially minimise the risk for condylar avascular necrosis and nonunion²⁹. We adopted a typical posterolateral/posteromedial approach (described by Henderson) for lateral and medial Hoffa fractures, respectively, and achieved satisfactory results. Both surgical approaches allow direct access to the articular aspect of the fracture and avoid injury to the arterial network of the patella and extensor mechanism of the knee, which sometimes occurs using the medial parapatellar approach32-34.

Many studies have shown that stabilisation after the completion of reduction can be accomplished with two cancellous screws placed anteroposteriorly or posteroanteriorly^{3,18,26-29,34-36}. However, a biomechanical study has shown that posteroanterior screws have a stronger resistance to shearing force than anteroposterior screws³⁷. However, posteroanterior screws need to be countersunk, which may damage the cartilage. Therefore, the use of headless screws for Hoffa fractures was described as an alternative in a previous case report³⁸. However, the drawbacks for headless screws are their small size and length, and more than two screws are required for stabilisation.

Although two cancellous screws with diameters of either 3.5 mm or 6.5 mm are generally considered sufficient for stabilizing isolated Hoffa fractures, several cases of fixation failure with cancellous screws have been reported^{31,39}. In Hoffa fractures, the fragment is continually exposed to physiological shearing stresses in the sagittal plane during normal flexion/extension. Varus/valgus stress may also exist in the coronal plane, even though the collateral ligaments are intact. The cancellous screws can perhaps only provide interfragmentary compression; there is no available hardware to resist the powerful physiological shearing stress. Therefore, to buttress the fragments against the shearing stress, two 3.5 mm posteroanterior screws were implanted. Meanwhile, we placed a common reconstruction plate between the affected femoral condyle and the distal femur for every patient. In our series, 6 of the 7 patients achieved anatomical reduction, and 1 achieved acceptable reduction with this fixation method. At the final follow up, there was no loss of reduction or failure of fixation. The final knee rating was excellent in 3 patients and good in 3 patients, and the satisfaction rate reached 85.7%. We believe that the reconstruction plate combination with posteroanterior screws can efficiently neutralise the shearing stresses caused by normal flexion/extension of the varus/valgus of the knee joint and ultimately obtain satisfactory clinical results.

Isolated Hoffa fractures are rare intra-articular injuries, and the diagnosis can often be missed. A high index of suspicion is needed to avoid missing these fractures. CT should be an important part of making an exact diagnosis and a preoperative plan. The typical posterolateral/posteromedial approaches described in our study can provide direct visualisation of the fracture and articular surface, which is necessary for achieving a perfect anatomic reduction and fixation. Internal fixation using a common reconstruction plate combination with cancellous screws can achieve a stable and rigid fixation and obtain a good functional outcome in the long term. However, the limitations of the study, including its non-prospective nature, the lack of controls and the small sample size, are difficult to overcome due to the rarity of the fracture.

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Trauma scores and oxidant factor levels in trauma patients: Clinical controlled study oxidant factors in trauma patients

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Abstract

Objective: Trauma scores are used for survey probability and severity of injuries in patients. This study aims to investigate the degree to Revised Trauma Score (RTS), that is significant in terms of determining the severity of injuries and analyzing the correlation between Total Oxidant Status (TOS) and Total Antioxidant Status (TAS).

Methods: 30 adults with severe trauma, 30 adults with light trauma among patients who refered to emergency clinic over a 1-year period and 30 individuals were involved. Age,sex, vital signs,resumes,injured organ,type of trauma and results were recorded.RTS scores were identified for all the patients and their blood was drawn for analysis of TOS and TAS.

Results: The average age of the patients is 41.6 and male-female rate was 1/4.4. The most common trauma type was motor vehicle accident (43.3%), and the most common part of the body exposed to trauma is head and neck with the percentage of 41.7%. The average of TOS and Oxidatif Stress Index (OSI) of the cases is found to be higher in comparison with the control group and TAS scores are stated to be in a lower degree.

Conclusion: We found an increment in Free Oxygen Radicals (FOR) from the results of TOS and OSI. RTS may have an effect on TOS and TAS levels.

Key words: Revised Trauma Score, Total Oxidant Status, Total Antioxidant Status.

Introduction

Trauma is a problem that effects especially young population negatively and therefore causes production loss along with health problems in many countries. Various trauma scores are used for the assessment of survey probability and severity of injuries in trauma patients. Glasgow Coma Scale (GCS) that is used for evaluating head trauma and consciousness; Trauma Score (TS) for physiological dimension of trauma and Revised Trauma Score (RTS) for improving TS are some of the trauma scores utilizing trauma incidents (1-4). The Revised Trauma Score is a physiological scoring system with high inter-rater reliability and demonstrated accurracy in predicting death. It is scored from the first set of data obtained on the patient, and consists of Glasgow Coma Scala, Systolic Blood Pressure (SBP) and Respiratory Rate [(RR) (5) (Table 1)].

The RTS (GCS+ SBP+RR) is heavily weighted towards the Glasgow Coma Scale to compensate for major head injury without multisystem injury or major physiological changes. A threshold of RTS<4 has been proposed to identify those patients who should be treated in a trauma centre, although this value might be a little low. The RTS

Table 1. Revised Trauma Score, Coded- Revised Trauma Score

| Glasgow Coma Scale (GCS) | Systolic Blood Pressure(SBP) | Respiratory Rate(RR) | Coded Value |
|--------------------------|------------------------------|----------------------|-------------|
| 13-15 | >89 | 10-29 | 4 |
| 9-12 | 76-89 | >29 | 3 |
| 6-8 | 50-75 | 6-9 | 2 |
| 4-5 | 1-49 | 1-5 | 1 |
| 3 | 0 | 0 | 0 |

correlates well with the probability of survival (6) (Figure 1).



Figure 1. Survival Probability by Coded-Revised Trauma Score

There are two forms of RTS: First one is the simple form and used commonly around the world for triage and is called Triage-RTS (T-RTS). However, there are some defects in this scoring system (7). Second form is used for evaluating patient death and named as Coded RTS (0,9368 (GCS) + 0, 7326 (SBP) + 0,2908 (RR). It is identified that all parameters in RTS have no significance in same ratio for evaluating death, so different significance codes were measured for each parameter. Coded RTS values vary between 0 (the worst prognosis) and 7,84 (the best prognosis). If a patient has a C-RTS 4 or less, patient must be evaluated in a trauma center (8).

Trauma causes oxidative stress as an effect of ROS (Reactive Oxygen Species) that is produced in trauma cases more than in physiologic conditions. Oxidative Stress is the imbalance between the free radicals such as oxygen, nitrogen and antioxidant mechanism detoxifying them in terms of reactive oxygen types. This oxidation injury involves cell and tissue damage. Shock evolving as a result of trauma in the organism causes impairment of metabolic balance between tissues, metabolisms of carbohydrate, protein and lipid.

Reactive oxygen species are charged in etiology of enfective complications and multiple organ failure syndrome (9). There is Erel method standartized for determining grade of oxidative stress in clinical assessment. Oxidant and antioxidant capacities of the cells might be measured by this method. TAS (Total antioxidant status): This is a fully automatic method measuring total antioxidant capacity of the body against free radicals (10,11). Labor principle, Fe2+-o-dianisidin complex forms OH radical reacting with hydrogene peroxide by Fenton-type reaction. These powerful reactive oxygen species are demoted and react with colorless o-dianisidin molecule by forming yellow-brown dianisidil radicals at low pH. Dianisidil radicals join advanced oxidation reactions and increase color formation, but antioxidants in samples stop color formation by suppressing oxidation reactions. This reaction is evaluated spectrophotometrically in automatic analyzer. TOS (Total oxidant status): This is a fully automatic colorimetric method (10). Oxidants in samples oxidize complex of ferrous ion-o-dianisidin to ferric ion. Glyserol accelerates and triples this reaction. Ferric ions form a colorful comlex with xylenol orange at asidic pH. Color is related to amount of oxidants in sample and may be measured spectrophotometrically. OSI (Oxidative Stress Index): TOS / TAS x 100. Index between TOS and TAS.

According to WHO (World Health Organisation) an accident means 'already unplanned and unexpected event that could be concluded as injury' (12). As determined by WHO in 'The Goals of Health for Everyone in 21th Century ' 5 major pandemias are reported that plans to be reduced gradually in worldwide up to year of 2020 in Goal 3. One of the pandemias is accidents (13). Serum/plasma concentrations of various oxidants might be measured separately, but these measurements are expensive, need more time and labor and require more complicated technics. Therefore measurement of various oxidant molecules one by one is not practical. Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) of a sample are measurable by aggregating their oxidant and antioxidant effects (14). Determining oxidative stress level through measuring is an effective cautionary signal in the way of forming an opinion about tissue damage and the precautions to be taken.

This study aims to investigate the degree to RTS is significant in terms of determining the severity of injuries and analyzing the correlation between TOS and TAS.

Materials and methods

60 adult trauma patients who admitted to our emergency department between the dates January 1st and December 31st, 2009 were included in the study. All patients were evaluated and ressusited according to Advanced Trauma Life Support program, diagnosed and treated with respect to existing protocols. Age, sex, vital signs (respiration count, arterial blood pressure, pulse), resumes, trauma scores (GCS, RTS), type of trauma, trauma location and conclusions of patients were recorded.

The proportion of patients excluded under each criterion was as follows; 1-Patients with chronic illnesses (diabetes mellitus, hypertension, hyperlipidemia, hyperthyroidism, chronic cardiac failure, renal failure, chronic liver disease, chronic lung illnesses, neurologic disease, malignancy), 2-constant drug disposers, alcohol or cigarette addicts, 3-regular antioxidant disposers, 4-pregnants, 5-trauma patients younger than 18 years old, 6-morbidly obese patients.

30 patients with severe trauma (GCS \leq 8), 30 patients with mild trauma (GCS > 9) and 30 healthy volunteers involved in the study (control group). 8 cc blood was drawn to tubes. Blood was centrifuged at 5000 rpm for 15 minutes in NUVE branded centrifugal device. At the same day samples were put into the deep freeze at -80° C until the analysis day. Then, their spectrophotometric analyzes were done in Perkin Elmer branded (UV/ Vis spectrophotometer model lambda 20 – USA) spectrophotometric device. TOS and TAS measures were taken by TOS and TAS direct measurement kits (TAS, TOS Rel Assay- Turkey) involved by Erel in 2004.

Primarily, three different spectrophotometer tubs were used for spectrophotometric measurements and 800 μ l TAS reagent 1 (R1) was put into them. 50 μ L standart 1, standart 2 and sample were added. Then, in spectrometer at 660 nm first absorbents were recorded. After that, 125 μ l TAS reagent 2 (R2) was added into it and it was left in room temperature for ten miutes. Finally ,again, at 660 nm second absorbents were recorded. The same procedures were applied for TOS measurements also, however only TOS reagant 1 and 2 were used instead.

Data were analyzed in Statistical Package for Social Sciences (SPSS) 15.0 tool for statistical assessment. Analytic comparisons were done by using Two Independent Groups T Test. Pearson Correlation Test was used for correlation analyzes. p<0.05 was accepted for significance value.

Results

Age interval was 18-89 years, the average age of the patients was 41,6. Male-female ratio was 1/4. The most common trauma type was motor vehicle accident (43.3%), and the most common part of the body exposed to trauma was head and neck with the percentage of 41.7% (Table 2).

Table 2. Dispersion of trauma patients accordingto the part of the body exposed to trauma

| Trauma location | Number | % |
|-----------------|--------|-------|
| Head-neck | 25 | 41,7 |
| Thorax | 3 | 5,0 |
| Abdomen | 2 | 3,3 |
| Extremity | 4 | 6,6 |
| Other * | 26 | 43,4 |
| Total | 60 | 100,0 |

*: more than one trauma location for a patient

18 patients (30%) were admitted to the intensive care unit and 18 patients (30%) were admitted to other services. 10 patients (16.7 %) were discharged from the hospital and five patients with the percentage of 8.3% died (Table 3).

Table 3. Dispersion of patients for emergency service results

| Result | Number | % |
|-----------|--------|-------|
| ICU | 18 | 30,0 |
| Service | 18 | 30,0 |
| Exitus | 5 | 8,3 |
| Discharge | 10 | 16,7 |
| Other* | 9 | 15,0 |
| Total | 60 | 100,0 |

*: desertion of emergency service without permission.

The average of TOS and OSI of the cases consulting to the service with total trauma patients was found to be higher in comparison with the control group and TAS scores were stated to be in a lower degree (Table 4). So, we can say that trauma caused oxidant stress in patients.

The average RTS of mild trauma patients (7.7 ± 0.3 trolox-eqv./l) was found to be higher and so meaningful in comparison with severe trauma patients (4.1 \pm 1.7 trolox-eqv./l) (p: 0.000). The average of TOS and OSI results of the patients with severe trauma was higher compared to the patients with light trauma and their RTS and TAS scores were found to be lower (Table 5). So, we found

that severe trauma affected patients much more than mild trauma by producing oxidant radicals.

In comparison of RTS results to TOS, TAS and OSI in severe trauma and normal patients, it was observed that while RTS results were decreasing, namely in severe trauma, TOS increased, and the relation between them was meaningful (r: -0.324, p: 0.002); TAS decreased and the relation between them was found to be meaningful (r: -0.575, p: 0.000); OSI increased and the relation between them was statistically significant (r: -0.324, p: 0.002) (Table 6).

Discussion

In the present study, it is aimed to investigate the degree to what extend RTS is significant in

determining the severity of injuries and prognosis and analyze the correlation between that significance rate, TOS and TAS that are considered to vary in trauma cases.

This difference might be derived from men who work outside and who are exposed to multiple trauma more than women, which is consistent with results from the previous studies (15). When comparing trauma types, motor vehicle accidents with 26 (43,3%) cases were the most common trauma type. In a previous study of Boullion and friends', also motor vehicle accidents were the most common cause of traumas. Gradually increase of trauma exposion in developing countries may be based on population increase, as well as pervading driving motor vehicles and all of these provide inadequate groundwork, though (16,17,18,19). Our study showed the most common trauma type was motor vehicle accident (43.3%).

Head trauma composed up to half of death along with trauma in our study in parallel to the literature (20, 21). Head trauma incidence is reported as 1-2% in literature (22, 23). In our study head trauma incidence was found to be 41,7%. In our opinion, the most common parts of the body exposed to trauma were head and neck with the percentage of 41.7%.

Results of TOS and OSI that were evaluated spectrophotometrically for assessment of Free Oxygen Radicals (FOR) in adult trauma patients confirmed increase in production of FOR in our study. There are few studies in literature reporting increment in production of FOR in experimental animal models (24,25,26).The average RTS of mild trauma patients (7.7 ± 0.2 trolox-eqv./l) was

| 1 5 | | 1 | 0 1 |
|---------|--------------------------------------|--------------------------------------|------------------|
| Groups | TOS Av. ± s.d (trolox-eqv./lt) | TAS Av. ± s.d (trolox-eqv./lt) | OSI Av. ± s.d |
| Trauma | 1.62 ± 0.03 | 1.61 ± 0.04 | 100.9 ± 3.4 |
| Control | 1.61 ± 0.02 | 1.64 ± 0.03 | 98.0 ± 1.1 |
| Р | 0.109 | 0.0001 | 0.0001 |

Table 4. Comparison of TOS, TAS and OSI in Total Trauma patients and Control groups

| Table 5. Comparison of RTS, TOS, TAS and OSI in severe trauma and mild trauma gro | oups |
|---|------|
|---|------|

| Groups | RTS Av. ± s.d | TOS Av. ± s.d (trolox-eqv./lt) | TAS Av. ± s.d (trolox-eqv./lt) | OSI Av. ± s.d |
|---------------|------------------|--------------------------------------|--------------------------------------|------------------|
| Severe Trauma | 4.1 ± 1.7 | 1.64 ± 0.03 | 1.58 ± 0.01 | 103.6 ± 1.5 |
| Mild Trauma | 7.7 ± 0.3 | 1.61 ± 0.02 | 1.64 ± 0.04 | 98.1±2.5 |
| P< | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

| Table 6. | Comparison | of RTS, | TOS, | TAS and | OSI | in | severe | trauma | and | control | groups |
|----------|------------|---------|------|---------|-----|----|--------|--------|-----|---------|--------|
|----------|------------|---------|------|---------|-----|----|--------|--------|-----|---------|--------|

| Groups | RTS Av. ± s.d | TOS Av. ± s.d (trolox-eqv./lt) | TAS Av. ± s.d (trolox-eqv./lt) | OSI Av. ± s.d |
|---------------|------------------|--------------------------------------|--------------------------------------|------------------|
| Severe Trauma | 4.10 ± 1.7 | 1.64 ± 0.03 | 1.58 ± 0.01 | 103.6 ± 1.5 |
| Control | 7.84 ± 0.0 | 1.61 ± 0.02 | 1.64 ± 0.03 | 98.0± 1.1 |
| Р | 0.0001 | 0.002 | 0.0001 | 0.0001 |

found to be higher and meaningful in compared to severe trauma patients (4.1±1.7 trolox-eqv./l) (p<0.0001). The average of TOS and OSI of the cases consulting to the service with trauma was found to be higher in comparison to the control group and TAS scores were stated to be in a lower degree. At the same time, the average of TOS and OSI results of the patients with severe trauma was higher compared to the patients with light trauma and their RTS and TAS scores were found to be lower. These comparisons were statistically significant (p<0.0001). In the present study, we stated that GCS and RTS have an effect on TOS and TAS levels. On the basis of these findings, it is maintained that for trauma cases TOS and TAS scores are more effective providing objective analysis instead of trauma scores which is a way of subjective measurement.

Conclusion

In conclusion, commonly used trauma scores usually evaluate patients by the current vital findings and physical examination findings, therefore this data could be subjective. Namely, whether arterial blood pressure of an untreatened hipertensive patient is usually 170/100 mmHg and if decreased to 120/80 mmHg according to bleeding after trauma, it is not right to accept him/her as a normotensive patient due to trauma scores. So, using an objective assessment criterion that is not affected by prior physiologic conditions of patients and advise severity of trauma by changing due to intensity of trauma is going to be effective in providing insights into prognosis and follow-up of patients.

This study is one of the first models to develop a clinical prediction for oxidant status among ED patients evaluated for trauma. We showed an increase in Free Oxygen Radicals (FOR) from the results of TOS and OSI. We stated that RTS has an effect on TOS and TAS levels.

Limitations

Serious trauma patients might have additional illnesses but they were unconscious patients, so we could report their illnesses only up to declarations of their relatives. In addition, this study was conducted at a single tertiary referral center ED, which might introduce selection and referral bias and limit applicability to patients treated in other settings. Another limitation is the number of patients, which might be increased in further studies.

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The efficacy of adefovir dipivoxil in chronic hepatitis B patients with lamivudine resistance

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Abstract

Background/aims: The aims of this study were to evaluate the efficacy of adefovir dipivoxil monotherapy against lamivudine-resistant chronic hepatitis B and to compare hepatitis B e antigen (HBeAg) positive and anti-HBe positive patients at baseline for response to adefovir dipivoxil treatment in our patients.

Methods: In total, 47 Turkish chronic hepatitis B patients with clinical lamivudine resistance were enrolled, including 22 HBeAg positive patients and 25 HBeAg negative patients who met the inclusion criteria in this retrospective study. The follow-up period of patients treated with only adefovir dipivoxil at 10 mg daily as an antiviral treatment was a mean 12.95 months (range 6-24). The serum hepatitis B virus-DNA, alanine aminotransferase levels, and HBeAg/anti-HBe status in HBeAg positive patients were evaluated at baseline 3, 6, 12, 18 and 24 months during the treatment period.

Results: There were significant changes in mean hepatitis B virus-DNA and alanine aminotransferase levels following 3, 6, 12, 18 and 24 months of treatment with adefovir dipivoxil from baseline (p<0.001). The percentage of change was similar in alanine aminotransferase and hepatitis B virus-DNA levels in both HBeAg-positive and anti-HBe-positive groups, but was not statistically significant. HBeAg/anti-HBe seroconversion did not occur in any HBeAg-positive patients.

Conclusions: The results of this study demonstrate that a mean 12.95 months (range 6-24 months) of adefovir dipivoxil monotherapy in lamivudine-resistant chronic hepatitis B patients is effective in both HBeAg and anti-HBe positive patients in our region.

Key words: Adefovir dipivoxil, lamivudine, resistance, chronic hepatitis B, HBV.

Introduction

An estimated 400 million persons worldwide are chronically infected with the hepatitis B virus (HBV) (1). Chronic hepatitis B (CHB) is a proven risk factor for the development of liver cirrhosis and hepatocellular carcinoma (HCC) (2). CHB should be treated effectively to prevent the progression to cirrhosis, HCC and death. Currently available therapeutic options are standard interferon, pegylated interferon, and nucleotide and nucleoside analogues for CHB (3). Nucleotide and nucleoside analogues suppress HBV replication in most patients and improve transaminase levels and liver histology (4,5). Lamivudine (LAM) is the first commonly used nucleoside analog approved for the treatment of CHB. However, LAM is associated with the highly frequent emergence of drug resistant mutants (6). YMDD (tyrosine-methionine-aspartate-aspartate) mutation develops in about 15% of patients per year and in 70% after 5 years of lamivudine monotherapy (7-9). LAMresistant HBV may cause hepatitis flares, hepatic decompensation and death (10,11).

Adefovir dipivoxil (ADV) is an adenine nucleotide analogue used in the treatment of CHB in adults with hepatitis B viral replication (12,13). ADV is effective against both wild-type and lamivudine-resistant HBV (14,15). In lamivudineresistant patients receiving LAM treatment with the addition of ADV or adefovir alone, virologic and biochemical improvements have been observed (16,17). The efficacy of the drugs was affected both by the virus and host factors. At the time of the present study, interferon, LAM and ADV have been approved for the treatment of chronic HBV infection in Turkey.

In the present study, we aimed to evaluate the efficacy of ADV monotherapy in Turkish CHB patients with LAM resistance and to compare the

HBeAg positive and anti-HBe positive patients for response to treatment.

Materials and Methods

Study subjects

This study was performed by retrospective chart review. Forty-seven adult CHB Turkish patients (22 HBeAg positive, 25 anti HBe positive) with clinical LAM resistance who visited the outpatient clinic of the Gastroenterology Department of the Uludag University Medical School from January 2004 to December 2006 and met the inclusion criteria were enrolled in this study. The inclusion criteria were as follows: positive for hepatitis B surface antigen (HBsAg) for at least 6 months and with a history of LAM therapy; alanine aminotransferase (ALT) levels between 2-to-10 times the upper normal levels; HBV DNA levels $\geq 10^4$ copies/mL. Patients were excluded if coinfected with HIV and other hepatitis viruses, nonalcoholic fatty liver disease, alcoholic liver disease, decompensated liver cirrhosis, hepatocellular carcinoma, liver transplantation, or impaired renal function (serum creatinine >1.5 mg/dL); or were taking concomitant immunosuppressant drugs and in the last 6 months used interferon, immunoglobulin or any other antiviral therapy demonstrating anti-HBV activity. Resistance to LAM is defined as clinical in the presence of higher viral replication (HBV-DNA \geq 10⁴ copies/mL) and an increase in ALT values (ALT > 1.5 upper limit of normal) while patients continue LAM treatment (18,19). Resistance to LAM was researched as genotypic in 19 patients of 47 and the YMDD mutation was found in all of them.

Baseline factors including age, gender, body mass index (BMI), duration of LAM use,

HBeAg status, ALT and DNA levels were analyzed. All patients were given ADV 10 mg daily as antiviral treatment for at least 6 months (range, 6-24 months). During the treatment period, patients were not given any other antiviral drugs and medicines for liver function protection. All patients were followed-up every 3 months by biochemical and virologic assessments to determine the efficacy of ADV. In addition, anti-HBe positive and HBeAg positive patients at baseline were compared for response to ADV treatment.

Serum assays and definitions

Analyses of serum levels of ALT were performed at baseline, and months 3, 6, 12, 18 and 24 of ADV treatment, using the autoanalyzer (Aeroset System Abbott, Abbott Laboratories, Diagnostic Division, IL, USA). The status of HBsAg, HBeAg and antibodies to HBeAg (anti-HBe) was measured by a microparticle enzyme-linked immunosorbent assay at baseline and months 6, 12, 18, and 24. Serum HBV DNA was quantified by a real-time polymerase chain reaction (Corbett Research, Rotor-Gene 2000/3000, Australia; detection limit 400 copies/mL) at baseline and months 3, 6, 12, 18, 24. The upper limit of normal ALT was defined as 43UI/L. All assays were performed in microbiological laboratories at the Uludağ University, School of Medicine.

The following definitions are used: Virological response (VR) (undetectable HBV DNA [HBV-DNA <400 copies/mL]), partial virologic response (PVR) (residual HBV DNA level less than $< 10^4$ copies/mL), virological breakthrough (a confirmed increase in HBV DNA level of more than 1 log10 copies/mL compared to the nadir [lowest value] HBV DNA level on therapy), primary non-response (PNR) (less than 1log10 copies/mL decrease in HBV DNA level from baseline at 3 months of therapy), biochemical response (BR) (normalization of ALT levels [<43UI/L]), serological response (disappearance of HBeAg with or without the appearance of HBeAg in HBeAg positive patients) according to EASL Clinical Practice Guidelines of 2009 (20). The occurrence of resistance to ADV was defined as the emergence of a virological breakthrough and an increase in serum ALT values compared to the treatment nadir that was confirmed in 2 consecutive tests.

Statistical Analysis

The SPSS (Statistical Package for the Social Sciences, for Windows, release 12.0.0 standard version) software was used for statistical evaluations. Quantitative data were presented as the mean \pm standard deviation. Significance was defined as <0.05. Categorical data was evaluated using chi-square analysis or with Pearson's correlation as appropriate. Continuous data were analyzed with the Mann-Whitney U test and the Wilcoxon signed rank test for paired analyses.

Baseline characteristics

A total of 47 patients were included, consisting of 34 males and 13 females, with ages ranging from 18 to 64 years (mean, 42.5 ± 13.3 years) and a BMI ranging from 18 to 43 (mean, 25.89 ± 4.1). The median duration of LAM treatment was 32.2 \pm 17.2 months (range, 9-96 months). The baseline data of patients were as follows: the mean level of HBV DNA was $7.57 \pm 8.07 \log 10$ copies/mL and the mean level of ALT was 111.15 ± 70.29 IU/mL. The comparison of the HBeAg positive group (n=22) and anti-HBe positive group (n=25)with gender, BMI and duration of lamivudine use found no statistically significant differences. Comparison of the two groups was statistically significant in terms of age (p = 0.004) (Table 1). The durations of patient follow-up were 22 patients for 6 months, 19 patients for 12 months, 17 patients for 18 months and 9 patients for 24 months in the HBeAg-positive group; and 25 patients for Table 1. Comparison of the baseline characteristics of HbeAg positive anti Hbe positive groups

6 months, 22 patients for 12 months, 14 patients for 18 months and 10 patients for 24 months in the anti-HBe-positive group.

Virological and Biochemical Effect

The comparison of the mean level of HBV DNA at baseline and at months 3, 6, 12, 18 and 24 months after treatment with ADV showed statistically significant differences (p<0.0001, p<0.0001, <0.0001, p<0.0001, p=0.001, respectively). Similarly, the comparison of the mean level of ALT at baseline and at months 3, 6, 12, 18 and 24 after treatment with ADV showed statistically significant differences (p<0.0001, p<0.0001, p<0.0001, p<0.0001, p=0.003, respectively) (Table 2).

Comparison of HbeAg positive and anti-Hbe positive groups

In both groups, the percentage change was similar in ALT and HBV-DNA levels at months 3, 6, 12, 18 and 24 after treatment with ADV. These results were not statistically significant (Table 3, 4).

| | HBeAg positive group (n=22) | Anti HBe positive group (n=25) | P value |
|--------------------------|-----------------------------|--------------------------------|---------|
| Gender (male/female) | 18/7 | 16/6 | 0.60 |
| Age (years) | 36.7 ± 14.1 | 47.6 ± 10.3 | 0.004 |
| BMI | 26 ± 5.25 | 25.80 ± 2.85 | 0.87 |
| LAM-Tx duration (months) | 35.9 ± 20 | 28.9 ± 14 | 0.16 |

LAM: Lamivudine, Tx: Treatment, BMI: Body mass index.

Table 2. Comparison of mean levels of HBV-DNA (log10 copies/mL) and ALT (UI/L) at baseline and months 3, 6, 12, 18 and 24 after treatment with ADV (Mean \pm SD)

| | HBV-DNA (log10 copies/mL) | P value | ALT (UI/L) | P value |
|------------------|---------------------------|----------|------------------|----------|
| Baseline (n=47) | 7.57 ± 8.07 | | 111.1 ± 70.2 | |
| Months 3 (n=47) | 6.60 ± 7.38 | < 0.0001 | 59.4 ± 24.4 | < 0.0001 |
| Months 6 (n=47) | 6.42 ± 7.19 | < 0.0001 | 52.1 ± 25.5 | < 0.0001 |
| Months 12 (n=41) | 6.96 ± 7.62 | < 0.0001 | 47.6 ± 37.3 | < 0.0001 |
| Months 18 (n=31) | 7.22 ± 7.90 | < 0.0001 | 52.8 ± 46.6 | < 0.0001 |
| Months 24 (n=19) | 6.42 ± 7.05 | 0.001 | 50.1 ± 59.5 | 0.003 |

ALT: Alanine aminotransferase, SD: standard deviation, ADV: Adefovir dipivoxil.

Table 3. The percentage change in ALT levels at baseline and months 3, 6, 12, 18, 24 after treatment with ADV (Mean percentage change \pm SD)

| Months | HBeAg positive group (n=22) | Anti-HBe positive group (n=25) | P value |
|--------|-----------------------------|--------------------------------|---------|
| 3 | -0.28 ± 0.34 | -0.42 ± 0.26 | 0.14 |
| 6 | -0.33 ± 0.30 | -0.54 ± 0.26 | 0.08 |
| 12 | -0.32 ± 0.63 | -0.54 ± 0.39 | 0.05 |
| 18 | -0.31 ± 0.64 | -0.37 ± 0.88 | 0.11 |
| 24 | -0.45 ± 0.22 | -0.35 ± 0.94 | 0.24 |

ALT: Alanine aminotransferase, SD: standard deviation, ADV: Adefovir dipivoxil.

| Months | HBeAg positive group (n=22) | Anti-HBe positive group (n=25) | P value |
|--------|-----------------------------|--------------------------------|---------|
| 3 | -0.84 ± 0.27 | -0.74 ± 0.73 | 0.85 |
| 6 | -0.85 ± 0.29 | -0.88 ± 0.29 | 0.78 |
| 12 | -0.78 ± 0.50 | -0.91 ± 0.25 | 0.97 |
| 18 | -0.78 ± 0.40 | -0.83 ± 0.34 | 0.57 |
| 24 | -0.93 ± 0.17 | -0.26 ± 3.74 | 0.35 |

Table 4. The percentage change in HBV-DNA levels at baseline and months 3, 6, 12, 18, 24 after treatment with ADV (Mean percentage change \pm *SD)*

ALT: Alanine aminotransferase, SD: standard deviation, ADV: Adefovir dipivoxil.

| | Months 3 n=47 (%rate) | Months 6 n=47 (%rate) | Months 12 n=41 (%rate) | Months 18 n=31 (%rate) | Months 24 n=19 (%rate) |
|-----|--------------------------|--------------------------|---------------------------|---------------------------|---------------------------|
| VR | 3 (%6.3) | 10 (%21.2) | 16 (%39) | 11 (%35.4) | 9 (%47.3) |
| PVR | 18 (%38.2) | 27 (%57.4) | 30 (%73.1) | 21 (%67.7) | 14 (%73.6) |
| PNR | 26 (%55.3) | 17 (%36.1) | 6 (%16.6) | 6 (%19.3) | 2 (%10.5) |
| BR | 13 (%27.6) | 20 (%42.5) | 26 (%63.4) | 18 (%58) | 10 (%52.6) |

Table 5. Virological and biochemical response to adefovir therapy

VR: Virological response (undetectable HBV DNA [HBV-DNA <400 copies/mL]). PVR: Partial virologic response (residual HBV DNA level less than < 10^4 copies/mL). PNR: Primary non-response (less than 1log10 copies/mL decrease in HBV DNA level from baseline at 3 months of therapy). BR: Biochemical response (normalization of ALT levels [<43UI/L]).

Serological effect and safety profile

One of the 22 patients who was initially HBeAg positive lost HBeAg at 6 months and remained HBeAg negative and anti-HBe negative up to 24 months. HBeAg/anti-HBe seroconversion did not occur in any HBeAg positive patients. ADV treatment was safe and well tolerated. No patient discontinued the ADV during the study period. A serious adverse event was not recorded. The number of patients observed for virological response, partial virologic response, biochemical response and primary non-response is given in Table 5. No patient showed resistance to ADV, virological breakthrough, hepatitis flare or hepatic decompensation during the ADV treatment.

Discussion

Currently treatment options for CHB patients are using interferon, and nucleotide and nucleoside analogues. In several large, multicenter controlled trials, lamivudine therapy has been shown to induce improvements in the virologic, biochemical and histologic features in CHB patients (21,22). However the emergence of lamivudine-resistant mutations is important as they reduce the therapeutic effects and may lead to a biochemical and virologic rebound. LAM-resistant mutations have been reported to occur in approximately 70% of patients after 4–5 years of LAM treatment (6,23,24). LAM resistance may lead to severe complications such as hepatitis flare or hepatic decompensation (25). Therefore it is necessary to treat LAM-resistant patients with other nucleoside or nucleotide analogues. Previous studies have shown that the ADV is effective in CHB patients with LAM-resistant mutants. The resistance profile of ADV is better than that of LAM (12,13,17,26).

In the present study, ADV alone was used for a mean 12.95 months (range 6-24 months) to treat CHB patients with LAM-resistant mutants in Turkey. VR was observed in 6.3% (3/47), 21.2% (10/47), 39% (16/41), 35.4% (11/31) and 47.3% (9/19) of patients at months 3, 6, 12, 18 and 24, respectively, in our study. These rates of VR are lower than several previous studies such as one study that found that 70% of 54 LAM-resistant CHB patients achieved virological response during the first year of ADV treatment (27). However, while VR was defined as HBV-DNA levels <104 copies/ml in some previous studies, we defined PVR a value according to the EASL Clinical Practice Guidelines of 2009. In our study, PVR was observed in 38.2% (18/47), 57.4% (27/47), 73.1% (30/41), 67.7%(21/31) and 73.6% (14/19) of patients at months 3, 6, 12, 18 and 24, respectively. Our results are in accordance with other investigators who also presented similar rates of VR and PVR (28). Antiviral efficacy of ADV increased with treatment duration as seen in the rates of PVR.

In the present study, BR was observed in 27.6% (13/47), 42.5% (20/47), 63.4% (26/41), 58% (18/31) and 52.6% (10/19) of patients at months 3, 6, 12, 18 and 24, and PNR was observed in 55.3% (26/47), 36.1% (17/47), 16.6% (6/41), 19.6% (6/31) and 10.5% (2/19) of patients at months 3, 6, 12, 18 and 24, respectively. There was no significant difference in VR, PVR and BR between HBeAg positive and anti-HBeAg positive patients. These results are also similar to the results of previous studies (28,33,36).

One of the 22 patients who was initially HBeAg positive lost HBeAg at 6 months and remained HBeAg negative / anti-HBe negative for up to 24 months. HBeAg/anti-HBe seroconversion did not occur in any HBeAg positive patients in our study. In another study, adefovir salvage treatment was administered to LAM-resistant CHB patients, and the results showed HBeAg loss rates of 10.7%, within the first year of therapy (27).

No patient showed resistance to ADV, virological breakthrough, hepatitis flare or hepatic decompensation during the ADV treatment in our study. In the previous two studies, researchers did not find genotypic resistance to ADV at 48 weeks in 11 of 30 CHB patients with confirmed LAM-resistant HBV (29,30). The emergence of ADV-resistant mutations increases in proportion to the duration of treatment. However, adefovir resistance in lamivudine resistant patients is more frequent than in naiv patients, as shown in different studies (31,32). Lee JM et al. reported that the overall cumulative virological response rate at 5 years of adefovir dipivoxil therapy was 48.8% in 320 LAM-resistant CHB patients and the 5-year cumulative probability of genotypic resistance and virological breakthrough was 65.6% and 61.8%, respectively (33).

It has been reported that ADV decreases renal function (13). In our study, serum creatinine levels of all patients were not increased and a serious adverse event was not seen during the treatment period.

Some researchers compared LAM and ADV combination therapy with ADV monotherapy after LAM-induced viral genetic resistance. They showed that LAM and ADV combination therapy

is a more effective treatment to get a virological response and has lower genetic resistance than ADV monotherapy (9,34,35). According to a meta-analysis that included six trials with 442 patients, the combination with LAM performed better than adefovir monotherapy with respect to HBV DNA suppression and the development of adefovir resistance in LAM-resistant CHB patients (37). Similarly, a current comparative metaanalysis of ADV monotherapy and combination therapy of ADV and LAM for LAM-resistant chronic hepatitis supports this finding (36). Recent studies suggest that the addition of ADV to LAM could prevent the emergence of ADV resistance in LAM-resistant patients, particularly when ADV therapy is initiated as early as possible.

Current AASLD guidelines recommend the addition of ADV or tenofovir to LAM treatment in patients with LAM resistance (38). The European Association for the Study of the Liver (EASL) guidelines recommend the addition of tenofovir in LAM-resistant patients (add ADV if tenofovir is not yet available) (20). At the time of the present study, tenofovir has not been approved for the treatment of chronic HBV infection in Turkey.

Limitations of this study are the short duration of patient follow-up and small number of patients. In addition, there was no combination treatment group to compare efficacy in the present study.

In conclusion, the results of this study demonstrate that a mean 12.95 months (range 6-24 months) of ADV monotherapy in LAM-resistant CHB patients is effective in both HBeAg and anti-HBe positive patients in our region.

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Clinical research of Ultrasound-guided percutaneous radiofrequency ablation for benign thyroid nodules

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Abstract

Purpose: To investigate the clinical effectiveness of ultrasound- guided percutaneous ablation for benign thyroid nodules.

Method: A total of 72 benign cold thyroid nodules in 40 patients were investigated. Shrinkage and elastosonographic characteristics in the ablated areas were evaluated. Thyroid function and complications related to ablation were monitored during the follow-up period (mean 6.15 months).

Results: 72 of the ablation areas completely covered the size of nodules. An ultrasound followup found that ablation areas softened gradually, and were inordinately absorbed. Twenty of the nodules regressed completely. The volume-reduction ratio (VRR) was 84.3% at six months. Three patients showed transient hyperthyroidism. No severe complications were observed.

Conclusion: Ultrasound-guided percutaneous radiofrequency ablation is effective, minimally invasive, and controllable for treating benign thyroid nodules.

Key word: Ultrasound-guide, thyroid nodul, radiofrequency ablation.

Introduction

The occurrence of benign thyroid nodules is common. The incidence of malignant transformation is %. The growth of benign thyroid nodules could affect other organs and give the patients a variety of psychological pressure. So many patients seek surgical treatment. In addition to the relatively greater damage, there is a lot of risk in surgery. It has been reported that the recurrence rate is as high as 10%-30%[1]. More complications may arise if the surgery has to be repeated. Recently, ultrasound (US)-guided radiofrequency ablation (RFA) for benign thyroid nodules has been under investigation. The purpose of our study was to evaluate the clinical effectiveness of US-guided percutaneous radiofrequency ablation for benign thyroid nodules.

Materials and Methods

Ethical Considerations

All the authors confirm that this study comply with the guidelines and obtain informed consent from all the patients.

Patients

A total of 72 benign thyroid nodules in 45 patients were treated with US-guided RFA between February 2010 and November 2011. The study population was composed of 9 male and 36 female patients, aged 15-84 (mean age,44.75±12.62 years). The sizes of the 72 nodules ranged from 5-30mm. The volumes were 0.04-22.49 ml. Written informed consent was obtained from all patients.

The inclusion criteria was as follows: (1) The nodule was diagnosed as benign by fine needle aspiration biopsy(FNAB) and needle Biopsy (2). The largest dimension of the nodule was more than 10mm.(3) The sizes of the nodules ranged from 5-10mm, but the patients had psychological pressure and wanted to be treated. (4) The thyroid function and blood coagulation of patient were in the normal range.

Pre-ablation Evaluation

Before ablation, laboratory studies including coagulation, thyroid function (TSH, FT3, FT4, TG, A-TG, A-TPO), and virological examination were performed with all patients. All US examinations were performed with a 5-13 MHZ linear probe on a real-time US system (HI VISION Preirus).The size, composition, intra-nodular vascularity, elastosonographic characteristics, and locations were evaluated. Three orthogonal diameters of the tumors were considered: the largest diameter and two other perpendicular ones were measured. The volume of the tumor was calculated by the following equation: $V = \pi abc/6$ (V: volume, a: the largest diameter, b and c: the two perpendicular diameters).

RF ablation

The patients were placed in the supine position with the neck extended. Under the US examination, investigators determined the approach route for the electrode. An RF generator(Celon AG medical instruments, Germany)and Single needle bipolar type with 1-cm active tip(Celon ProBreath, Germany) were used. All US procedures were performed with the same transducer using the freehand technique. The patients were treated with 2% lidocaine for local anesthesia of the puncture site and around the thyroid gland. A mixture of lidocaine and saline was injected around the thyroid to protect trachea, esophagus, neck vessels, and recurrent laryngeal nervefrom being injured [2].

The catheter needle was inserted into the thyroid nodule under US guidance along the transverse of the nodule. The needle core was removed and the electrode tip was inserted, followed by ablation. While the ablation system was running, the area of the nodule became a transit hyperechoic zone. Once the hyperechoic area covered the nodule, an alarm sounded to alert completion of the ablation. If the nodule was so large that it needed more treatment time, the electrode tip was positioned in the deepest and most remote part of the nodule. The direction of ablation went from deep to shallow, and from distal to proximal. If the composition of nodule was mixed, we aspirated the fluid as much as possible before the electrode tip was inserted. Ablation began with 5 W of power. If the patients could not tolerate the pain, we decreased the power to 4W.

Follow-up evaluation

After RF ablation, US examination, US examinations were performed at 1, 3, 6 and 12 months (range, 1–20 months). The size was measured and the volume of the tumor was calculated with the pre experiment parameters. The VRR was calculated by the following equation: volume reduction ratio (%, VRR) = {[initial volume (ml) –final

volume (ml)] $\times 100$ }/initial volume. Intra-nodular vascularity and elastosonographic characteristics were also evaluated. The thyroid function test (TFT) was carried out one week after ablation. If the TFT was abnormal, the test was carried out every other month until the result was normal.

Any complications during or after the procedure were also assessed by clinical signs and symptoms.

All studies have been approved by the ethics committee of the First Affiliated Hospital of Dalian Medical University and carried out under the institutional guidelines.

Results

44 patients were investigated out of which there was one mortality. The follow-up period was 1-20 months with a median of 6.15 months. Follow-up US examination revealed that the intra-nodular vascularity of the treated nodules had been destroyed.

After six months, 45.9% (17/37) of the nodules VRR was greater than 50%, 54,1% of the treated nodules had disappeared, there were two ablation zones that had disappeared after three months. There were no nodules that presented with an increased volume after ablation at the last follow-up.

Over time, the ablation zones softened gradually, and the grade of the ultrsound-elastosonography (US-E) reduced[3] .Elastosonography showed that 11/54(19.28%) had an ES 1-2 at the one month follow-up, and 11/14(81.48%) at six months. 43/54(79.63%) had an ES 3-4 at the one month follow-up, and only 3/14(21.43) had an ES 3 at six months. Ablation areas soften gradually with time

One week after ablation, thyroid function tests were performed on all patients.6.7% (3/45) patients had hyperthyroidism ((TSH: 0.02IU/ml, FT3: 7.55pmol/L, FT4: 26.29pmol/L; TSH: 0.06IU/ml, FT3: 8.5pmol/L, FT4: 23.25pmol/L; TSH: 0.206IU/ ml, FT3: 4.9pmol/L, FT4: 17.23pmol/L), with varying degrees of clinical symptoms. However these patients did normalize by the one month follow-up. Among patients there was subclinical hyperthyroidism with A-TG increase. The thyroid function of the other patients was within the normal range.

There were no serious complications such as esophageal or trachea injury, or skin burn. During the ablation, hematoma developed in two patients. We did not ablate the nodule residue until 48 hours after the hematoma was absorbed. One patient complained of hoarseness immediately after ablation, but recovered within three months without further treatment. All patients complained of mild pain, which subsided the next day. While the fibrous capsule of thyroid gland was separated, patients complained of voice changes and throat discomfort, both of which eased within hours to days.

Discussion

With the popularization of high resolution ultrasound, the detection rate of thyroid nodules elevated from 19% to 67%, with 5% \sim 15% of patients presenting a malignancy [4]. Although the malignant transformation rate of benign nodules is low, patients generally choose aggressive treatment, especially for Hashimoto's thyroiditis, which according to reports has a carcinoma rate of 27.0% [5]. Currently, the main treatment method is surgery, but there are some drawbacks. The rate of permanent injury of the recurrent laryngeal nerve is $2.0\% \sim 13.0\%$ [6]. Moreover, there is a cosmetic problem. The traditional minimally invasive treatment is ethanol sclerotherapy. The effect on solid nodules is poorer than with cystic ones, because the dispersion of ethanol is asymmetrical. Poor control of ethanol liquidity was common around tissue adhesion and hindered further nodule therapy. Since Kim et al [7] first reported radiofrequency ablation for thyroid benign nodules in 2006, this minimally invasive therapy has wide application prospects in the treatment of thyroid benign nodules.

In our study, the mean VRR was 70.9% 84.3% at three months and six months respectively. It is reported that the mean VRR ranged from 50.7%-84.8% at the six month follow-up [7-9]. In this study, 39.02%(16/41) of nodules had disappeared at the six month follow-up, among these, two cases disappeared at the three month follow-up. It was discovered that the mean volume reduction of cases with Hashimoto's thyroiditis was higher compared to the other forms of thyroiditis. In this study, there were five cases with Hashimoto's thyroiditis: one patient's symptoms vanished by the three months follow-up and the others showed the same improvement by the six month follow-up. We suggest that because the thyroid tissue around the ablation zone has a rich blood supply this expedites the removal of necrotic tissue. We did not find an influence factor in terms of volume reduction, such as age, or the composition of nodule and vascularity. We did find that the speed of volume reduction is faster at six months than the other follow-up periods. Therefore it is suggested that a six month follow-up is a significant time point for evaluating the effectiveness of ablation.

We suggest that hydrolase released by coagulation necrosis of the ablation lesion and neutrophils liquefies and dissolves the necrotic tissue, which are absorbed by lymphatic vessels and blood vessels causing the volume of RF ablation lesion to decrease and the tissue soften.

At the one week follow-up, 93.22% patient's thyroid function was normal, 4 patients' (6.78%) thyroid function changed. Three patients had hyperthyroidism with no symptoms. At one month, the thyroid function of these three patients was normal without any treatment, which was consistent with the results of Cakir et al.[10-13]. It was suggested that this maybe due to the effects of thyroid compensation. We did not find any influencing factor for this. There was one patient with subclinical hyperthyroidism presenting with high anti-thyroglobulin antibodies (A-TG), which was normalized within a month, this might because of the immune responses caused by partial denaturation of thyroglobulin. [14] Kim et al[7] had reported one case with high TSH but there was no case in our study.

No major complications were observed. After RFA differential extent of swelling pain was reported, but all patients recovered spontaneously without squealae in a few days. Hematoma around the thyroid gland was found in the two early cases during the operation puncture. We suggest if the ultrasound showed difficulty in removing a vessel away from the puncture path, injected 1% lidocaine was used to remove it. If the node was on the back of thyroid gland and near recurrent nerve, an injection of 0.5% lidocaine was used to separate the thyroid back capsule and gap between trachea and esophagus. Throat discomfort, voice change and tissue appeared from post-RFA within a few hours to a few days. This was because lidocaine had a temporal effect on the recurrent nerve and it recovered spontaneously after few minutes and hours. Quickly and accurately puncturing, and keeping the needle top up to increase the distance

between the node helped to avoid injury when injected 0.5% lidocaine. This was because the anatomical structure around the thyroid gland was loose and lidocaine was rapidly absorbed.

Conclusion

Ultrasound-guided percutaneous radiofrequency ablation is effective minimally invasive, and controllable for treating benign thyroid nodules.

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Can Cesarean rates be reduced?

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Abstract

Aim: The aim of this study was to investigate the change in cesarean delivery rates over the years, the distribution of indications and safety measures which can be taken to reduce caesarean rates, in the light of the literature.

Material and methods: Vaginal and cesarean deliveries in Nenehatun Obstetrics and Gynecology Hospital between January 2009 and December 2012 were retrospectively analyzed.

Results: During this four-year period, 33520 deliveries occured. Of these, 21516 patients delivered vaginally while 12004 delivered by cesarean section. In 2009, 3250 (38.9%) patients were performed cesarean section while in 2012, 2917 (34.4%) patients had a cesarean delivery. The most common indications of cesarean procedures were previous uterine surgery (5398/45%), fetal distress (1458/ 12.1%) and cephalopelvic disproportion (1261/ 10.5%), respectively. During the period from 2009 until 2012, whilst there was a statistically significant increase in the rate of cesarean sections performed for the indication of previous uterine surgery (p=0.001), we observed a significant decrease in the indication rates of fetal distres (p=0,001), cephalopelvic disproportion (p=0,001), prolonged labor (p=0,001) and elective cesarean (p=0,001). There was no significant change in the rates of other indications. A decrease in the overall rate of cesarean sections was observed over the years.

Conclusion: Cesarean rates in our hospital tended to decrease, as rates fell from 38.9% in 2009 to 34.4% in 2012. A striking finding in our study was that at the end of the 4-year period indication of previous uterine surgery doubled despite the decline of the overall rates of cesarean sections. One reason for this was reduction in primary cesarean rates due to stricter decision-making for the primary cesarean indications according to the national health policy regulations. In addition, another effective measure to be taken in order to reduce rates of cesarean sections is encouraging vaginal birth after cesarean (VBAC) in women with history of previous cesarean section.

Key words: Cesarean, vaginal delivery, indications, uterine surgery.

Introduction

Increasing cesarean rates around the world has become one of the most debated topics in obstetrics. High cesarean delivery rates do not seem to provide better perinatal care (1,2).

While reported cesarean rates in the U.S. and U.K. were 29.1% and 21.5%, respectively, those rates were reported as around 40% in Latin American countries. In Turkey, although cesarean delivery rates were reported as 21.2% according to the Turkish Population and Health Research conducted in 2003, actual rates seem to reach up to 40% according to up-to-date delivery statistics in some regions. This rate exceeds the target rate set by the World Health Organization (which is 10-15%) (3).

Cesarean section sould be considered as a major operation due to possible complications such as bleeding requiring blood transfusion, postoperative infection and thromboembolic events. Psychological, social, financial factors including advanced surgical and anesthetic techniques, defensive medicine due to medico-legal situations, advanced maternal age, and changing health care policies are considered as responsible from the increased rates of cesarean deliveries (4-6). Today, many countries are engaged in various efforts to reduce the rate of cesarean sections (7). The two most important indications for reducing cesarean rates appears to be prior cesarean delivery and breech presentation. However, uterine scar rupture, which is associated with maternal and fetal mortality risk of vaginal birth after cesarean (VBAC), is often a deterrent factor for obstetricians (8).

The aim of this study was to investigate the change in the rates of cesarean deliveries over the years, the distribution of indications and safety measures which can be taken to reduce caesarean rates, in the light of the literature.

We retrospectively analyzed the medical records of patients who had vaginal or cesarean delivery in Nenehatun Obstetrics and Gynecology Hospital between January 2009 and December 2012. Electronic archive of the hospital was used for the retrieval of patient information. Cases of vaginal and cesarean deliveries were evaluated separately by years. Annual distribution of cesarean rates and their indications and annual changes in the rates of cesarean delivery were analyzed. SPSS 15.0 software was used for statistical analysis of data. Statistical analysis of the change in the indications of cesarean sections by each year was performed by using the chi-square test. P <0.05 was considered as significant.

Results

A total of 33520 births took place in our hospital between January 2009 and December 2012. Out of these, 21516 delivered vaginally while 12004 were delivered by cesarean (Table 1). Mean maternal age was 28.7±5.1 years. Mean gestational age was 36.3 weeks and mean birth weight was 2953±723 grams. Indications for cesarean sections according to years were shown in table 2.

In our study, the main indications for cesarean section were previous uterine surgery, fetal distress, cephalopelvic disproportion (CPD), breech presentation, prolonged labor, multiple pregnancy, fetal macrosomy, placental pathologies (placenta previa, placental abruption), preeclampsia and elective (Table 2). Among 12004 cesarean cases during the4-year period, indications of cesarean procedures in order of frequency were previous uterine surgery (5398/45%), fetal distress (1458/ 12.1%), CPD (1261/ 10.5%), breech presentation (1190/9.9%), prolonged labor (1009/8.4%), multiple pregnancy (357/3%), fetal macrosomy (294/ 2.4%), placental pathologies (150/ 1.2%), cord prolapse (78/ 0.65%), preeclampsia (53/ 0.4%), elective (53/0.4%) and others (703/5.9%). As cesarean indications were evaluated according to the years, following conclusions were reached. During the period from 2009 until 2012, whilst there was a statistically significant increase in the rate of cesarean sections performed for the indication of previous uterine surgery (p=0.001), we observed a significant decrease in the indication rates of fe-

Table 1. Distribution of vaginal and cesarean deliveries for each study year

| Voor | Vaginal delivery | | Cesa | Total | |
|-------|------------------|------|-------|-------|-------|
| rear | n | % | n | % | n |
| 2009 | 5105 | 61.1 | 3250 | 38.9 | 8355 |
| 2010 | 5053 | 60.7 | 3276 | 39.3 | 8329 |
| 2011 | 5807 | 69.4 | 2561 | 30.6 | 8368 |
| 2012 | 5551 | 65.6 | 2917 | 34.4 | 8468 |
| Total | 21516 | 64.2 | 12004 | 35.8 | 33520 |

| Total | 21516 | 64.2 | 12004 | 3: |
|-------------------|------------------|-------------------|---------------|----|
| Table 2. Distribu | tion of cesarean | indications accor | ding to years | |

| | 0 | | | | | | | | | |
|----------------------|-----|------|------|------|------|------|------|-----|------|------|
| Indications | 20 | 09 | 20 | 10 | 20 | 11 | 20 | 12 | То | tal |
| Indications | n | % | n | % | n | % | n | % | n | % |
| Prev.Uterine surgery | 974 | 30 | 1169 | 35.7 | 1503 | 58.7 | 1752 | 60 | 5398 | 45 |
| Fetal distress | 568 | 17.5 | 397 | 12.1 | 239 | 9.3 | 254 | 8.7 | 1458 | 12.1 |
| CPD | 490 | 15.1 | 520 | 15.9 | 121 | 4.7 | 130 | 4.5 | 1261 | 10.5 |
| Breech presentation | 319 | 9.8 | 331 | 10.1 | 256 | 10 | 284 | 9.7 | 1190 | 9.9 |
| Prolonged labor | 328 | 10.1 | 348 | 10.6 | 171 | 6.7 | 162 | 5.6 | 1009 | 8.4 |
| Multiple pregnancy | 90 | 2.8 | 86 | 2.6 | 82 | 3.2 | 99 | 3.4 | 357 | 3 |
| Fetal macrosomy | 80 | 2.5 | 84 | 2.6 | 64 | 2.5 | 66 | 2.3 | 294 | 2.4 |
| Placental pathology | 42 | 1.3 | 40 | 1.2 | 29 | 1.1 | 39 | 1.3 | 150 | 1.2 |
| Cord prolapse | 25 | 0.7 | 17 | 0.5 | 17 | 0.7 | 19 | 0.7 | 78 | 0.6 |
| Preeclampsia | 11 | 0.3 | 17 | 0.5 | 8 | 0.3 | 170 | 0.6 | 53 | 0.4 |
| Elective | 28 | 0.8 | 24 | 0.7 | 1 | 0.04 | 0 | 0 | 53 | 0.4 |
| Others | 295 | 9.1 | 243 | 7.4 | 70 | 2.7 | 95 | 3.3 | 703 | 5.9 |

tal distres (p=0,001), cephalopelvic disproportion (p=0,001), prolonged labor (p=0,001) and elective cesarean (p=0,001). There was no significant change in the rates of other indications.

Discussion

Increased caesarean section rates in developed and developing countries has become an international major public health problem. While cesarean rate in 1970s was 5-7%, it increased up to 25-30% in 2003 (9). Cesarean section is a major abdominal surgery and a life-saving procedure for the mother and fetus in cases where complication and mortality risk of vaginal birth is high. However, increased cesarean rate is associated with both higher use of postpartum antibiotherapy and increased maternal mortality and fetal morbidity by 7 to 10 times compared to vaginal delivery.

Optimal cesarean rate recommended by World Health Organisation is 10-15% (3). Many previous studies which analyzed cesarean rates showed an increasing trend over years (10-13). In an earlier study, we found the cesarean section rate as 37.7% in 2002 and 51% in 2007, reaching a total cesarean section rate of 43.7% within those six-year period (14). In contrast to the above-mentioned studies, in the present study, we found a decreasing trend in cesarean rates in our hospital. We detected the cesarean rates to decrease from 38.9% in 2009 to 34.4% in 2012.

Showing an unpreventable increase over years, cesarean rates were reported as 29.1% in the U.S., 21.5% in the U.K., 27.3% in Asian countries and more than 50% in Brazil (4, 5, 15). In a large-scale population study in which cesarean rate was detected as 27.3%, Lumbiganon et al. reported an increased maternal morbidity and mortality index associated with cesarean by 2.7-14.5 times compared to the vaginal delivery (15). Similarly Villar et al. reported in their study including 8 countries in Latin America that median cesarean rate was 33% and increasing cesarean rates were correlated with both fetal mortality and mortality and mortality (5).

Many studies revealed that previous uterine surgery (previous cesarean, myomectomy etc.) was the most common indication for cesarean section (10-13). Yumru et al. detected the most common cesarean indication as previous uterine surgery (32.6%) (16). Simsek et al. also reported that the most common indication for cesarean was previous cesarean delivery (32.5%) (13). In a previous study, we had found previous cesarean delivery as comprising 37.8% of cesarean indications. While this rate was 35.6% in 2002, we detected it as 39.4% in 2006. In this study too, we detected that the most common indication for cesarean delivery in our hospital was previous uterine surgery. While previous uterine surgery comprised about 30% of cesarean indications in our hospital, it increases up to about 60% in 2012. One of the most important causes of the increasing rate of previous uterine surgery as an indication for cesarean section is that our hospital is a central obsterics hospital in Eastern Turkey which accepts many patient referrals from its periphery. Based on the findings from several retrospective studies, American College Of Obstetrics and Gynecology suggested in 2004 that in the presence of one previous low transverse cesarean delivery, clinically adequate pelvis, no other uterine scars or previous rupture, physician immediately available throughout active labor capable of monitoring labor and performing an emergency cesarean delivery, and availability of anesthesia and personnel for emergency cesarean delivery it may be reasonable to offer a trial of labor to women (17). It was reported that women who had one previous low tranverse cesarean delivery may have a safe vaginal delivery and risk of uterine rupture was 0.3% (18, 19). Today, previous cesatrean delivery is the most important cause of increasing cesarean rates and vaginal birth after cesarean should be encouraged in available women to control those rates (20). Additionally, women who had no previous cesarean section should be evaluated more meticulously when taking the decision of cesarean section.

Fetal distress was the second most frequent cesarean indication in our study. While Güney et al. reported the rate of fetal distress as 22.6%, Coskun et al. reported this rate as 13.8% (11,12). Yalınkaya et al. and Simsek et al. reported in their studies that the second most frequent cesarean indication was fetal distress (17.96% and 26.6%, respectively) (10-13). In a previous study we too, had detected the second most frequent cesarean indication as fetal distress (11.7%). Similarly, in the present study, we found the second most com-

mon indication for cesarean delivery as fetal distress (12.1%). Beginning from the early 1970s, as availability of electronic fetal monitoring became more widespread, number of therapeutic decisions based on the diagnosis of fetal distress increased.

Cephalopelvic disproportion was the third most frequent cesarean indication (10.5%) in our study. Diagnosis of true CPD is controversial. In many previous studies, it was reported that CPD could be predicted by external pelvimetry in 50% of cases (21,22). Remarkable proportion of women who had diagnosed as having CPD, had vaginal birth after cesarean in their future pregnancies. Overdiagnosis of CPD may result from other conditions such as medicolegal problems, epidural analgesia and health policies. While CPD as an indication of cesarean section was about 15% in 2009 and 2010, it was found in our study to decline in frequency, as around 4.5% within the last 2 years. Similarly, in 2011 and 2012 cesarean rates based on fetal distress, prolonged labor and elective indications showed a significant decrease. Possible reason for this decline was settling the indications of primary cesarean sections more meticulously in order to reduce cesarean rate of our hospital.

While cesarean rate for breech presentation was 30% in 1976, it reached 86% in 1996. As cases of breech presentation at term were well-selected, vaginal delivery was not associated with increased morbidity and mortality (23). Possibilities of difficult delivery of the after-coming head that may result in brachial plexus injury and cord entanglement or prolapse are the main factors for preferring cesarean section in breech presentation. While indication of breech presentation for cesarean section was reprted as 2.9% by Coskun et al., 8% by Guney et al., 9.2% by Simsek et al, we had found it as 10% in our previous study (11-14). In the present study, we found breech presentation as the 4th most frequent cesarean indication comprising 9.9% of all indications. Ventura et al. reported that they preferred cesarean section in 85% of all malpresentations including breech (24). In current practice, both modes of delivery are recognized as acceptable. Since breech vaginal delivery was reported to be associated with 3 times more frequent perinatal morbidity and mortality, we prefer cesarean section for breech presentations in our hospital (25).

Another frequently seen indication for cesarean section is the prolonged labor. Simsek et al. reported prolonged labor as the 3rd most common cesarean indication (18.9%) (13). In our study, this rate tended to decrease, as it was 10.1% in 2009 and 5.6% in 2012. Some studies suggested that the use of partogram for early diagnosis and timely management of abnormal labor patterns would be useful in reducing cesarean rates (26). Antenatal diagnosis and effective management of malpresentation largely prevents prolonged labor. Additionally, if oxtocin induction is administered in false labor or latent phase of labor, it may cause fetal distress and dysfunctional labor.

As a result, cesarean rates in our hospital tended to decrease, as the rate was 38.9% in 2009, it fell to 34.4% in 2012. Cesarean rates in our hospital are below rates reported in many studies in the literature (10-14). However, rates are still far from the target set by the World Health Organization (15%). One noteworthy result in this study was that, although previous uterine surgery as an indication for cesarean section almost doubled at the end of the 4-year period, overall cesarean rate declined. We suppose this was probably due to more meticulous application of indications for primary cesarean section, which we had suggested in our previous study as one of the measures to be taken in order to reduce cesarean section rates. Another measure to be taken in order to reduce rates of cesarean sections is to promote vaginal birth after cesarean in women under suitable conditions. According to the results of this study we suggest that as number of cesarean sections performed for the indication of previous cesarean decrease due to the decline in the rates of indications for primary cesarean section, overall cesarean rates will further fall.

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Evaluation of the risk for developing colon polyps malignancy

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Abstract

Most colorectal tumours develop from initially benign bowel polyps. The degeneration of adenomas which ultimately progress to malignant colorectal cancer (the adenoma-carcinoma sequence) is a process which may extend over a number of years. Causes of this degeneration include successive changes (mutations) to genes in the cells of the mucosal membrane of the bowel wall. These lead to a loss of the natural mechanisms which curb the uncontrolled growth of these cells, allowing them to spread as cancer cells.^(1,2,3)

Colorectal carcinomas are among the most frequent malignant diseases in western industrial countries. Colorectal cancer is the third most common cancer in both men and women in the United States. Every year, about 146.000 persons develop colorectal cancer, with about 50.000 deaths. Colorectal cancer incidence rates have been decreasing for most of the past two decades. This is a result of the increase in screening, that allows the detection and removal of colorectal polyps before they progress to cancer.

It is estimated that colorectal cancer can develop from an intestinal polyp within five to ten years. The risk of cancer increases with age. Most patients with colorectal cancer are over 50. In the course of time, certain cells inside the adenoma experience further gene mutations.

Most of colorectal tumours are benign epithelial polyps. The most common polyp, which can become an adenocarcinoma is the adenomatous polyp ^(1,2,47). Two of its features, size and grade of villousity, can predict the polyp's malignancy risk ^(1,5-8).

The second most common type of polyp is the hyperplastic polyp. By colonoscopic examination, it is impossible to distinguish these polyps from one another, therefore every colon polyp should be examined microscopically moreover, adenomatous tissue is found in 13% of hyperplastic polyps, also adenocarcioma foci are noticed within these areas. Consequently, the presence of hyperplastic polyps is an indicator of increased risk for colorectal cancer, although this type of polyp is not considered a neoplastic polyp⁽⁹⁻²⁰⁾.

Adenomas are macroscopically classified in pedunculated and sessile. Histologicaly they are classified as tubular, villous and tubulo-villous. According to their frequency, tubular adenomas are dominant. After WHO criteria, an adenoma is considered tubular if at least 80% of its glands are branched (tubular type), whereas it is considered a villous adenoma if at least 80% of glands are villiform ^(4,21-25).

Dysplastic polyps are also classified according to the degree of dysplasia, which is: low, medium or severe, based on structural and cytological characteristics. One polyp may contain foci with different degree of dysplasia; in these cases its classification is made by the most severe dysplastic focus it contains. Big adenomas, with villous structure, usually contain severe dysplasia. These adenomas are more likely to contain cancerous tissue ^(9,14).

The malignancy potential is higher among bigger adenomatous polyps, with villous structure and higher dysplastic degree.

Key words: colon polyps, malignancy, dysplastic degree.

Background and purpose

Given that colorectal polyps are a problem of great importance nowadays, we needed to define their frequency among our patients, their most common localization within colon, the most attacked age-group, as well as percentage of high-risk polyps, according to size, histologic type and degree of dysplasia. Estimating the importance of colonoscopic examination and early polypectomy in order of lowering the incidence of colorectal carcinoma is crucial aim of this study. Another objective of this study is the association of colorectal polyps with clinical symptoms, since indications for colonoscopy are usually defined by the patient's signs and symptoms. Some of our patients had nonspecific symptoms; on the other side, clinical symptoms like abdominal pain or rectorrhagia were sometimes consequences of another, accompanying pathology, like hemorrhoids or colitis.

Materials and methods

65 patients (of 1285 who underwent colonoscopy over a one-year period) with colon polyps detected during colonoscopy, entered this study. Polypectomy was performed with diathermy, while biopsy material was taken by forceps. Localization of each polyp was marked in centimeters as a distance from the anus – for polyps of rectum and sigma, and after segments of colon – for polyp's localized proximally from sigma. No sedatives or other medications were given before the examination. Colorectal polyps underwent polypectomy or biopsy - depending on risk for complications (hemorrhage, perforation, postpolypectomy syndrome), estimated by the endoscopist, according to the size of polyp, localization and fragility of mucosa. The material was fixed in formalin and underwent microscopic examination. Morphologically, polyps were categorized according to the degree of dysplasia. Adenomas with over 25% of villous structure are considered adenomas with villous component. Hyper plastic and inflamatory polyps were included in non-neoplastic polyps. Polyps were fixed with formaldehyde 10% and sent for histopathologic examination. They were categorized by the stage of dysplasia, according to the WHO classification. Adenomas with over 25% of villous structure are considered villous adenomas. Hyperplastic and inflamatory polyps are included in non-neoplastic polyps. The results underwent statistic analysis and were presented in form of datatables, graphs, as well as in other data presentation techniques.

Results

61-70 years of age was the most affected agegroup (by colorectal polyps), followed by 41-50 and 51-60 yr. (Table 1, Figure 1.)

Table 1. Colorectal polyps according to agegroups



Figure 1. Graphic presentation of colorectal polyps according to age-groups

Considering the median age, there were not found significant differences between men and women, but numerically men were dominant (Table 2, Figure 1.). *Table 2. Age of patients according to gender*

| Col. title | М | F |
|--------------------------|--------|---------|
| Sample size (N) | 45 | 20 |
| | | |
| Std. error of mean(SEM) | 2.038 | 2.665 |
| Lower 95% conf. limit | 51.734 | 48.671 |
| Upper 95% conf. limit | 59.955 | 59.829 |
| | | |
| Minimum | 9.000 | 33.000 |
| Median (50th percentile) | 58.000 | 53.000 |
| Maximum | 75.000 | 75.000 |
| | | |
| Normality test KS | 0.1262 | 0.09219 |
| Normality test P value | 0.0697 | >0.10 |
| Passed normality test? | Yes | Yes |

Analysis of results shows involvement of rectum and colon sigmoideum in majority of cases with polyps or neoplasm (Figure 2.)



Figure 2. Endoscopic diagnosis of polyps according to the involved segments of colon

Histopathologic analysis of colorectal polyps has shown that tubular adenoma is most common, followed by non-neoplastic and tubulo-villous polyps with dysplasia (figure 3).



Figure 3. Microscopic diagnosis of examined polyps



Figure 4. Clinical symptoms of patients with colorectal polyps

Discussion

Colorectal cancer can be prevented. Polypectomy of adenomatous polyps during colonoscopy stops their progression to cancer. Fecal occult blood test, sigmoidoscopy and colonoscopy are associated with lowering of colon cancer mortality. The incidence of colorectal cancer is also decreasing on the annual average of 1.5%, from 1985. Yet, much more can be done by including population in screening tests for colorectal cancer. More than 45% of patients are diagnosed with colorectal cancer in an inoperable stage. ^(33, 37)

It is estimated that during 2006 there has been more than 145 500 new cases with colorectal cancer and approximately 55 000 deaths from CRC in the USA, making this cancer the second cause of cancer death.³⁷ Colorectal adenomas, precursors of almost all sporadic CRC, are found in 40% of persons over 60 years of age. Nevertheless, not all colorectal polyps are adenomas, and not every adenoma is transformed to colorectal cancer. Since it is impossible to identify which polyp will be transformed to cancer, every polyp discovered during colonoscopy should be removed, or at least biopsied. Polypectomy avoids colorectal cancer risk.

In this study we have analyzed problems linked with malignant potential of colorectal polyps. From 65 patients with colorectal polyps, 49 resulted with adenomatous polyps (72.3%), of which 68.08% were tubular adenomas; tubulo-villous adenomas were 23%, and villous adenomas 8.51%. These results are in correlation with those from the literature.

After consulting different author's results, including ours, it is concluded that males are at higher risk for malignant transformation of colorectal polyps. Besides higher incidence of colon polyps in men (although both genders were represented with almost equal number of patients), dysplastic and villous polyps are also more frequent among them. Furthermore, the incidence of multiple polyps was higher in men: 13.85% of our patients had multiple polyps, or 9 patients, from which 8 were men and only one woman. ^(19, 22-34)

It is recognized that big (over 1 cm) and adenomatous polyps are high risk adenomas and their microscopic examination is mostly unfavorable. But in recent years, increasing attention is paid to small (6-10 mm) and very small (<6 mm) polyps, since there are found adenomas with villous components and dysplasia among them in considerable percentage. High risk adenomas have been considered adenomas greater than 10 mm, with villous architecture over 25% and adenomas with high grade dysplasia. When the concept of "advanced" or "dangerous" polyps was first described in 1992, some experts agreed that there was no need of treatment, not even follow up for small (diminutive) colorectal polyps. Now days, according to the published results of numerous research about diminutive polyps, there is no doubt that every colorectal polyp should be examined microscopically. "Polyps, even smaller than 6 mm may present an unfavourable histology, therefore they should be treated" - referring to the presentation of results from retrospective studies (presented in the annual meeting of The American Society of Colorectal Surgeons, held in New Orleans, Louisiana, on 25th of June 2003). "Even very small colorectal polyps cannot be ignored, since 4.5% of them resulted with high risk and 2 in 1000 were cancerous" - has concluded the author James M. Church, colorectal surgeon and head of Endoscopy Department in Clevelend Clinic foundation – Ohio, after a retrospective study of 5722 colorectal polyps. In our study, among polyps with villous components or with dysplasia, 34.78% were smaller than 5 mm, not including here polyps cathegorised as "small" (not exactly estimated) by the endoscopist, which would include 30.43% of these adenomas.⁽³⁸⁻⁵²⁾

The matter of clinical symptoms among patients with colorectal polyps is of a great importance, since they determine indications for colonoscopic examination. It is known from earlier publications that these patients may experience different symptoms, and this characteristic was also seen among our patients. In some cases, patients may have no symptoms, but colonoscopy is required for explaining an anaemia, or excluding a possible (malignant) pathology of this region. Often, symptoms are not a direct repercussion of the polyp, since its dimensions may be that small that can't interfere with normal transit in colon. In cases when patient experiences bleeding from the anal region, there are often also found haemorrhoids, which were cause of bleeding (and not the polyp). Conversely, there are situations in clinical practice, when patient's complains and the presence of blood in stools are attempted to be explained by the presence of haemorrhoids and colonoscopic examination is not required. But, this can be very dangerous and may carry the risk of late diagnosis of colorectal cancer, considering the frequent existence of haemorrhoids, simultaneously with colorectal polyps.(47-55)

Rectosigmoid is the most attacked region from colorectal polyps. Moreover, dysplastic and villous polyps are more frequent in this part of colon (approximately 80% of dysplastic and villous polyps are localised in this region). This is associated with determination of screening for early detection of polyps.⁽²⁵⁻³⁶⁾

Although molecular genetics of colorectal cancer has evolved in recent decades, only within recent years we began to understand epigenetic mechanisms which initiate and support growth of malignant tissue in colorectum. Similarly with genetic factors in tumour growth, informations form numerous research in colorectal cancer are utilised to elucidiate epigenetic mechanisms in other types of cancer.

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Being victimized and school alienation among elemantary school students in Zonguldak, Turkey

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Abstract

The aim of this study was to investigate the relation between being victimized and school alienation among elemantary school students in Zonguldak, Turkey. Besides in this study the relations between certain characteristics of students and being victimized and school alienation have been evalauted. The students (n = 820) were chosen from two elemantary schools in Zonguldak in the 2011–2012 academic year. Data have been collected via Student Information Form, Scale of School Alienation and Peer Victimization Scale. It was determineted that there were various associations between being victimized and school alienation and whether these associations vary by grade level, gender, school absenteeism, chronic disease and using prosthesis. According to the results of the study, it was suggested that prevention of being victimized and school alienation should become a priority issue for schools and prevention programs which involve whole school approach should be implemented in the schools.

Key words: students, bullying, victims, school alienation

Introduction

Bullying is the kind of words and actions that an individual faces by his/her peers throughout his/her development process, aiming to render deliberate and intentional physical, mental, social or psychological damage to the victim.¹⁻⁶ Bullying can have a repetitive character for a certain length of time, besides the victim falls into a position making it impossible for him/her to protect or defend himself/ herself.^{4,5} Bullying may be carried out by one individual or a group and can take many forms. Physical forms of bullying are mostly seen as beating,

pushing, pulling, slapping, hair or ear pulling, intentionally damaging physical objects; ⁵⁻⁹ emotional forms of bullying can be listed as nicknaming, mocking, humiliating, rumor spreading about the victim, excluding from the group, rejection to include victim into the game, victimizing to loneliness.^{5,6,8,9}

Bullying is a worldwide problem which reaches its highest rate in elementary school age and declines in adolescence and later.¹⁰ Study results showed a considerable variation, (4% to 45%), in the percentage of students who reported to have been victimized.^{5,8,11-16} However, bullying has been found to be associated with several physical, emotional, social and academic problems for victims.^{8,9,13,17-24} Several studies showed that victims exhibit greater psychological disorders, cultivate decreased self trust, develop worsened social relations, decreased school achievement and increased school absence.^{3,6,8,25-28}

In consequence of bullying, decreased academic achievement of students, increased school absence and even quitting school introduce the concept of "school alienation". In its widest definition "alienation" refers to an individual's entrapment with lonesome and desperation as a consequence of this person's diminishing coherence with society, culture and environment and worsened personal relations.^{29,30} "Alienation" is not a new concept. However, the studies focusing on "school alienation" are recent nonetheless in relevant studies it has been demonstrated that gender, educational background of parents, socio-economic level of parents and school, school types, classroom size and classroom level are all influential factors on alienation.³⁰⁻³¹ Although it is considered that being victimized may be directly related with students' school alienation no studies examining this relation have yet been ascertained. Therefore, this study was conducted to investigate the relation between being victimized and school alienation among elemantary school students in Zonguldak, Turkey. Besides in this study the relations between certain characteristics of students (attended school and class level, gender, number of siblings, what number the child is in the family, family type, school absence ratio, chronic disease and prosthesis use or not) and being victimized and school alienation have been evalauted. Based on the above literature review, the following hypotheses were tested in this study.

 H_1 : There is a relation between being victimized and school alienation.

H₂: There is a relation between certain characteristics of students and being victimized

H₃: There is a relationship between certain characteristics of students and school alienation

Material and Methods

Design and Sample

This cross-sectional study has been conducted in Zonguldak city center lying within Western Black Sea region of Turkey. Study population includes collectively 8736 elemantary school students (6th, 7th and 8th class levels) receiving education during 2011-2012 academic term. The number of students that would be included within sampling has been detected as 368 via the formula for calculating the sample with known universe. In the process of detecting students that would be integrated into sampling every elementary school within city center has been accepted as a cluster and a list of these schools has been created. From this list School A has been determined as the school where study would be executed via simple randomization method. There are total 428 students attending School A. Considering that there might be losses in the number of students that would be included in sampling throughout research period second school has been detected via simple randomization method as School B. There are collectively 489 students attending School B. Total 820 students from both schools have been included in study.

54.5% of students (n = 447) receive education in School B; 39.1% of students (n = 321) attend to 6th class, 28.4% (n = 233) attend to 7th class and 32.4% (n = 266) attend to 8th class. 51.5% (n=422) of students are boys; 12.8% (n=105) are single child, 34.1% (n = 280) are the first child of family. 73.7% (n = 604) of students have school absenteeism; 8.7% (n = 71) have chronic disease and 18.8% (n = 154) have prosthesis (Table 1-2).

| Table 1. Introa | luctory features | belonging to stude | ents |
|-----------------|------------------|--------------------|------|
|-----------------|------------------|--------------------|------|

| Variables | n | % | | |
|--------------------------|-----|------|--|--|
| Class level | | | | |
| 6 th class | 321 | 39.2 | | |
| 7 th class | 233 | 28.4 | | |
| 8 th class | 266 | 32.4 | | |
| Gender | | | | |
| Girl | 398 | 48.5 | | |
| Boy | 422 | 51.5 | | |
| Number of siblings | | | | |
| Single child | 105 | 12.8 | | |
| 2 siblings | 437 | 53.3 | | |
| 3 or more siblings | 278 | 33.9 | | |
| What number the child is | | | | |
| Single child | 105 | 12.8 | | |
| First child | 280 | 34.1 | | |
| Middle child | 99 | 12.1 | | |
| Last child | 336 | 41.0 | | |
| Family type | | | | |
| Nuclear family | 716 | 87.3 | | |
| Extended family | 64 | 7.8 | | |
| Disintegrated family | 40 | 4.9 | | |
| School absence ratio | | | | |
| Frequent absence | 604 | 73.7 | | |
| No absence | 216 | 26.3 | | |

Table 2. Health features of students

| Variables | n | % | | | |
|-------------------------|-----|-------|--|--|--|
| Chronic disease state* | | | | | |
| Has chronic disease | 71 | 8.7 | | | |
| No chronic disease | 749 | 91.3 | | | |
| State of prosthesis use | | | | | |
| Has prosthesis | 154 | 18.8 | | | |
| No prosthesis | 666 | 81.2 | | | |
| Total | 820 | 100.0 | | | |

Note *: *A disease that requires medication on a regular basis*

Instruments

Student Information Form (SIF). In this form there are 11 open-close ended questions evaluating students' personal and familial features, their school achievement and absence.

Peer Victimization Scale (PVS). The scale has been developed to identify Turkish children and adolescents who are exposed to peer bullying. This scale demands each participant to select for each

item the choices most appropriate as "never", "one time" and "several times". The answers are graded such: (2) points for "several times" (1) point for "one time" and (0) for "never". This scale which consists of five subdimensions as intimidating/suppression, mocking, open assault, relational attack and damaging personal belongings contains collectively 27 items. The highest score to achieve from the scale is 54, the lowest score is 0. High score received from scale indicates that this person is frequently target of peer attack while low score signifies that person is rarely or never a target. In addition to total score it is possible in this scale to separately calculate scores obtained from every single subdimension. Internal consistency coefficient of the scale has been detected as .86 for the entire scale.³²

Scale of School Alienation (SSA). Scale has been developed with the aim of measuring school alienation levels of elementary education students. This is a 5 Likert type self-evaluation scale ranging from choices "I completely agree" to "I completely disagree". In this scale which consists of four subdimensions as weakness, meaninglessness, social exclusion and normlessness there are collectively 14 items; 9 negative and 5 positive. The highest score to receive from the scale is 70, the lowest score is 14. High scores obtained from SSA indicate that students' level of school alienation is intense. Internal consistency coefficient of the scale has been determined for the entire scale as .80.³¹

Procedures

With the aim of using these scales in study, prior to collecting study data first written approval has been received from researchers who developed Peer Victimization Scale and Scale of School Alienation. In order to execute the study written approval has been received from Zonguldak Provincial Directorate of National Education. Subsequent to negotiating with school principals, appropriate dates and time to gather data have been selected. Students have been informed by researchers on the objective and significance of research. Students who agreed to participate in research have been distributed data gathering tools.

Data Analysis

Data obtained from Student Information Form have been identified as independent variables,

scores received from Peer Victimization Scale and Scale of School Alienation have been selected as dependent variables. In the evaluation of independent variables number and percentage values, in the comparison of scale scores with respect to independent variables Mann-Whitney U and Kruskal-Wallis tests, in the identification of the relation between two scales Spearman correlation analysis have been employed.

Results

The Relation Between Being Victimized and School Alienation

It has been set forth with this study that between being victimized and school alienation there is a positive- though weak- relation; the higher the ratio of being victimized the higher the level of students' school alienation is (p = 0.000, r = 0.278).

The Relation Between Certain Characteristics of Students and Being Victimized

It has been detected that in terms of being victimized there is no differentiation with respect to attended school and class level, gender, number of siblings, what number the child is in the family and family type (p > 0.05); but students who have school absenteeism (p = 0.004), who have a chronic disease (p = 0.013) and use prosthesis face higher ratios of bullying victimization (Table 3-4).

The Relation Between Certain Characteristics of Students and School Alienation

It has been detected that in terms of school alienation, there is no differentiation with respect to number of siblings, what number the child is in the family, family type and prosthesis use (p > 0.05). On the other hand it has been witnessed that with respect to class level there is a differentiation in terms of school alienation (p = 0.000), the differences cover all classes (p < 0.0167), the higher class level is (6th grade = 27.21 ± 8.74; 7th grade = 28.31 ± 7.87; 8th grade = 30.03 ± 7.67) the more alienated students are towards school. Furthermore it has been determined that students who have school absenteeism (p = 0.004), who have a chronic disease (p = 0.05) and whose gender is male (p = 0.000) feel more alienated towards school (Table 3-4).

| | Peer Victimization Scale (PVS) | | Scale of School Alienation (SSA) | |
|-----------------------|--------------------------------|---------------|----------------------------------|--------------|
| Variables (n=820) | _ | Median (Min., | _ | Median |
| | $X \pm SD$ | Max.) | $X \pm SD$ | (Min., Max.) |
| Class level | | | | |
| 6 th class | 5.82±7.00 | 3 (0, 42) | 27.21±8.74 | 25 (14, 65) |
| 7 th class | 5.97±6.88 | 4 (0, 38) | 28.31±7.87 | 26 (18, 57) |
| 8 th class | 6.48±7.12 | 4 (0, 49) | 30.03±7.67 | 29 (18, 62) |
| F | 2.6 | 31 | 29.154 | |
| р | 0.2 | .68 | 0.000 | |
| Gender | | | 1 | r |
| Girl | 5.58±6.23 | 4 (0, 41) | 27.00±7.69 | 25 (16, 62) |
| Boy | 6.55±7.64 | 4 (0, 49) | 29.79±8.51 | 29 (14, 65) |
| Z | -1.162 -5.145 | | -5.145 | |
| р | 0.2 | 45 | | 0.000 |
| Number of siblings | | | | |
| Single child | 6.33±6.62 | 4 (0, 29) | 28.43±8.26 | 26 (16, 50) |
| 2 siblings | 6.25±7.52 | 4 (0, 49) | 28.79±8.34 | 27 (14, 62) |
| 3 or more siblings | 5.72±6.26 | 4 (0, 37) | 27.88±8.07 | 26 (15, 65) |
| F | 0.6 | 52 | 2.372 | |
| р | 0.7 | 22 | | 0.305 |
| What number the child | l is | | | |
| Single child | 6.33±6.62 | 4 (0, 29) | 28.43±8.26 | 26 (16, 50) |
| First child | 6.30±7.27 | 4 (0, 41) | 28.56±8.47 | 27 (14, 65) |
| Middle child | 5.65±6.73 | 4 80, 37) | 27.16±7.35 | 25 (16, 44) |
| Last child | 5.94±6.99 | 4 (0, 49) | 28.71±8.28 | 27 (15, 62) |
| F | 0.6 | 99 | 2.563 | |
| p p | 0.705 0.464 | | | |
| Family type | | 4 (0, 10) | | |
| Nuclear family | 6.17±7.09 | 4 (0, 49) | 28.17±7.97 | 26 (14, 62) |
| Other | 5.42±6.34 | 3 (0, 32) | 30.26±9.73 | 29 (16, 65) |
| | -0.8 | 319 | -1.815 | |
| p p | 0.4 | 13 | | 0.070 |
| School absence ratio | 6.54.5.00 | 4 (0, 40) | 00.55.5.00 | 27 (14 (2)) |
| Frequent absence | 6.54±7.39 | 4 (0, 49) | 28.77±7.98 | 27 (14, 62) |
| No absence | 4.79±5.60 | 3 (0, 35) | 27.50±8.87 | 25 (16, 65) |
| | -2.8 | 387 | -2.878 | |
| р | 0.0 | 04 | | 0.004 |

Table 3. With respect to students' introductory features their state of exposure to victimization and school alienation

Note. Z = Value of Mann-Whitney U test, F = Value of Kruskal-Wallis test

Table 4. With respect to students' health features their state of exposure to victimization and school alienation

| | Peer Victimization Scale (PVS) | | Scale of School Alienation (OYÖ) | |
|-------------------------|--------------------------------|---------------------|----------------------------------|---------------------|
| Variables (n=820) | $\overline{X} \pm SD$ | Median (MinMax.) | $\overline{X} \pm SD$ | Median (MinMax.) |
| Chronic disease state | | | | |
| Has chronic disease | 8.46±9.41 | 6 (0, 42) | 30.04±8.06 | 29 (17, 53) |
| No chronic disease | 5.85±6.69 | 4 (0, 49) | 28.28±8.24 | 26 (14, 65) |
| Z | -2.473 | | -1.964 | |
| р | 0.013 | | 0.05 | |
| State of prosthesis use | | | | |
| Has prosthesis | 7.07±7.51 | 4 (0, 42) | 29.58±8.54 | 28 (18, 62) |
| No prosthesis | 5.85±6.87 | 4 (0, 49) | 28.17±8.15 | 26 (14, 65) |
| Z | -2.163 | | -1.904 | |
| р | 0.031 | | 0.057 | |

Note. Z = Value of Mann-Whitney U test

Discussion

It has been set forth in current paper that there is a positive-though weak-relation between victimization and school alienation. This relation bears importance since it points to the fact that as being victimized increases school alienation shall correspondingly rise. It has also been ascertained in this paper that students who have school absenteeism are, compared to the ones who attend school regularly, exposed to greater victimization thus suffer from deeper alienation. This finding drives us to consider that not only students who are victimized but also the ones who are entrapped in school alienation thrive to escape from their problems via school absence. Likewise previous studies have put forth that students who are victimized feel themselves academically inadequate, 3,27,28 that their school achievement degrades, they cultivate negative attitude towards school and their school absence ratio increases.^{3,6,25-28} However, negative relation students might experience with their peers might prevent them to feel themselves strong and safe thus it is considered that this negation might be a reason of alienation. Hence findings of current research bear importance since they manifest that being victimized alienates students towards school, it might bring with itself certain adverse reflections such as school absence and even leaving education half the way.

In current study it has also been put forth that with respect to class level there is no significant differentiation in terms of being victimized however it has also been seen that the higher the level of class is, the greater is being victimized. Aside from that it has been detected that the higher the level of class the greater is school alienation. Nonetheless in the rest of relevant studies, as opposed to this study findings, it has been seen that the higher the level of class the lesser is being victimized.^{17,33} Peer group is an environment where the student can get to know him/herself more objectively in comparison to familial environment and where s/ he can face realities from the lens of interpersonal relations. The influence of peer group on the child multiplies parallel to the age and this effect reaches its peak during adolescence period.25 The finding of current study manifesting that parallel to the rise in class level both being victimized and student's school alienation increase is compatible with findings of relevant literature.

It has been determined in this study that boy students are more victimized. On the other hand as relevant literature is analyzed it surfaces that with respect to gender variable there are different results related to being victimized. In some studies it has been stated that with respect to gender variable there is no difference in terms of being victimized,¹⁴ while some studies claim that boy students, 9,17,25,34,35 some researches claim that girl students^{5,33,36} face greater level of victimization. The variety of the bullying forms investigated might be crucial factors explaining the diversity in these findings. That is because while boy students can face mostly physical forms of bullying,^{8,32,36} girl students can be exposed to mostly emotional forms of bullying.^{32,36} In this study it has also been identified that compared to girl students boy students become more alienated towards school. An analysis of relevant literature has also provided differing results in terms of school alienation with respect to gender variable. Sanberk³⁷ claims that with respect to gender there is no difference in school alienation. Yigit³⁰ and Uzun-³¹reported that boy students; Celik²⁹ has pointed out that girl students become more alienated towards school. It is considered that these differences stem from the fact that students included in study scope belong to different stages of development. Indeed though it is possible to claim in studies focusing on high school students the differences in attitutidinal development of adolescents has no relation to gender variable, the same might not be hold true for elementary education students. That is related to the fact that in elementary education students it is well known that gender identity has a vital role and even play groups are formed from children of the same sex.³⁸ Thus it might be considered natural that attitudes of elementary education students vary with respect to gender. Consequently this might account for the reason why in this study school alienation has varied with respect to gender variable. Additionally the finding obtained hereby that boy students face greater levels of being victimized as well as school alienation bears importance as it supports the positive association between being victimized and school alienation.

A person's earliest social environment is his/ her family thus interpersonal relations within his/ her family, social features of family, the financial means they own and attitude developed in family play role in a child's development. Though the difference is insignificant, it has been identified that children with nuclear family, no siblings or being the oldest child despite having siblings are more victimized. Contrary to findings set forth in current study in other studies it has been detected that children with extended families, more than 3 or above siblings are more victimized.^{1,25} Each family has its own dynamics that is why the diversity in these findings might be attributed to the differences in family structures.

Physical appearance and features distinguishing the student from others might be a matter of humiliation amidst elementary school students. In a study investigating the causes of bullying amidst students the reasons have been listed such: presence of a scar / accident injury on body, extreme weakness in weight, looking physically powerless, extreme tallness, speaking disorders, being sick or diseased, extreme shortness.³³ The finding of current study claiming that students with chronic diseases or using prosthesis face significant level of victimization is supportive of relevant literature. Besides students afflicted with health problems might - due to the fear they feel they would be ostracized by friends or always lose because of this insufficient physical capacity- go through feelings of horror, anxiety, weakness and social exclusion. Likewise present study has manifested that students with a chronic disease or using prosthesis are more alienated towards school and this is a finding supportive of our previous assumption.

Limitations

The limitations of this study are that characteristics of bullies and places where bullying actions are performed have not been analyzed hereby. While preparing programs to prevent bullying, in terms of detecting the priorities of program it is crucial to identify risk group in terms of both victims and bullies, to designate the places bullying is most frequently seen within school borders, around school or places students spend most of their free time.

Conclusion

This study fills a gap in the literature through its examination of the various associations between being victimized and school alienation and whether these associations vary by grade level, gender, school absenteeism, chronic disease and using prosthesis.

Implications for Practice

The findings of this study have several implications for school practice. From the above results and discussion, the present study makes suggestions as follows:

- Prevention of bullying and school alienation should become a prioritized issue for schools.
- -The extension of this study within a sampling group covering all Turkey, detecting the places and characteristics of not only victims but bullies and places where bullying behaviors are exhibited might provide essential data in the preparation of a program addressing to prevent bullying and school alienation. The prevention program should involve a whole school approach. This program should include assessing the problem, planning school conference days, providing better supervision at recess, forming a prevention coordinating group, encouraging parentteacher meetings, establishing classroom rules against bullying, holding classroom meetings about bullying, requiring talks with victims and bullies, and scheduling talks with the parents of involved students.
- For those students who experience school alienation it might be useful to form study groups led by a psychological counselor to assist students in improving their social relations. If needed school principal, teachers and parents might also be invited to these sessions with the aim of strengthening inter communication.
- Since it is clear that school alienation rises parallel to the increase in being victimized, to the end of solidifying students' bonds with school, starting from preschool education it might be aidful to supply students with programs aiming to prevent bullying and school alienation.

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White-coat hypertension as a risk factor for the development of sustained hypertension

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Abstract

Background: White coat hypertension (WCH), a high blood pressure recording with normal ambulatory blood pressure monitoring (ABPM), is a relatively common observation in hypertensive subjects. The aim of this study was to evaluate the determinants of WCH in the general population and trace the 1 year follow up result.

Methods: Blood pressure (BP) was measured during four visits at 20 community pharmacies (three measurements per visit). Patients with WCH had a mean BP >140/90 mmHg and daytime ambulatory readings \leq 135/85 mmHg, whereas patients with sustained hypertension had a mean BP >140/90 mmHg and daytime ambulatory readings >135/85 mmHg.

Results: In total, 1838 subjects (mean age 66±10 years) were enrolled. Seven-hundred and ninety (43%) were normotensive, 183 (1%) had masked hypertension, of whom 460 (25%) had white-coat hypertension, and 570 (31%) had sustained hypertension. Patients with white coat hypertension were older, and had a higher body mass index, waist-hip ratio, office systolic (SBP) and diastolic BP (DBP), and albumin clearance ratio (p<0.001). Multivariate analysis revealed that age [odds ratio (OR)=1.029, 95% confident interval (CI)=1.001-1.057, p=0.04], office SBP (OR=1.145, 95% CI=1.119-1.172, p<0.001), and office DBP (OR=1.080, 95% CI=1.047-1.114, p<0.001) predict WCH. At a 1-year follow up, 10% (47/460) of subjects with WCH demonstrated sustained hypertension (p < 0.001).

Conclusions: WCH confers significantly less risk than sustained hypertension. WCH may be predicted by age, and higher office SBP and DBP. Our study supports the recommendation to perform BP measurements at home and to schedule regular follow-ups, as part of normal care in patients with WCH.

Key word: White-coat hypertension, sustained hypertension.

Introduction

Blood pressure (BP) measurements in the clinical setting may not reflect BP behavior away from the clinic¹. White-coat hypertension (WCH) is the most commonly used term to describe a BP response in individuals whose BP is elevated only in a medical setting. WCH has been reported in as many as 20% of patients in whom hypertension has been diagnosed via office BP2. BP measurements in the clinic may be incorrect for a number of reasons, including inaccuracies in the measurement technique and artificial increases in BP produced by the white-coat effect (WCE)³. Although WCH has a relatively benign outcome compared with sustained mild hypertension, failure to recognize WCH may lead to an inappropriate use of antihypertensive medications^{4, 5}. Unfortunately, patients with WCH have few characteristics other than elevated BP while in a medical environment. Further, patients with WCH appear to have more target organ damage than normotensive subjects^{6,7}. WCH was also found to be a risk factor for sustained hypertension⁸, insulin resistance⁹, and increased all-cause mortality¹⁰. The aim of this study was to evaluate the determinants of WCH in the general population.

Materials and Methods

Participants and study design

The study was a cross-sectional study on individuals aged >45 years conducted at 20 community pharmacies. BP was measured during four visits to a community pharmacy (three measurements per visit) and via home BP monitoring. A comprehensive patient history and physical exam was conducted for each subject. All subjects were monitored for pharmacy BP, home BP, body mass index, and waist and hip circumference, and blood samples were taken. Pharmacy and home BP were measured according to a standardized protocol by a trained nurse with an electronic BP monitor. Systolic (SBP) and diastolic BP (DBP) were measured in the morning after subjects had sat for 10 minutes in a quiet room. BP measurements were performed in triplicate and were carried out on the same arm separated by 30 second intervals. The average value of the last two measurements was recorded as the final BP. Blood samples were obtained following an overnight fast and prior to taking any medications. The BP measurement protocol was repeated 1 year later.

Diagnosis of white-coat hypertension

WCH was defined as a clinic BP>140/90 mmHg with a daytime mean BP < 135/85 mmHg at home.

Diagnosis of sustained hypertension

Sustained hypertension was defined as a clinic BP >140/90 mmHg with a daytime mean BP >135/85 mmHg at home.

Statistical Analysis

Continuous variables were presented as means \pm standard deviation (SD), and categorical variables were presented as frequencies and percentages. The independent t- and Chi-square tests were used for comparisons between groups. Furthermore, a stepwise multiple logistic regression analysis was performed to assess the clinical determinants between independent variables and left ventricular hypertrophy. For all tests, a *P*-value <0.05 was considered to indicate statistical significance.

Results

Patient characteristics

In total, 1838 individuals (mean age 66 ± 10 years) were enrolled in the study, with 62.6% being female. Normal BP was recorded in 790 (43%) subjects, 183 (1%) of whom had masked hypertension, while 460 (25%) were diagnosed with WCH,

| | Normotensive | Sustained Hypertension | White-coat hypertension | Masked hypertension | p-value |
|-----------------------|-----------------|---------------------------|----------------------------|-------------------------|---------|
| Number of subjects | 790 | 567 | 448 | 26 | |
| Age (years) | 63 ± 10 | $70\pm9^{\mathrm{a}}$ | $66 \pm 9b$ | 67 ± 10 | < 0.001 |
| Male (%) | 35 | 39 | 39 | 39 | 0.553 |
| Diabetes mellitus (%) | 8 | 22ª | 9 | 27° | < 0.001 |
| Tobacco use (%) | 17 | 17 | 15 | 19 | 0.193 |
| Dyslipidemia (%) | 9 | 18ª | 9 | 23° | < 0.001 |
| CVA | 1 | 2 | 1 | 0 | 0.301 |
| Gout | 2 | 4 | 2 | 4 | 0.257 |
| Tea | 35 | 34 | 34 | 27 | 0.844 |
| Alcohol | 13 | 15 | 16 | 19 | 0.081 |
| Exercise | 57 | 66ª | 60 | 54 | 0.007 |
| Body high (cm) | 157 ± 8 | 156 ± 8 | 157 ± 8 | 157 ± 8 | 0.098 |
| Body weight (kg) | 60 ± 10 | 63 ± 11^{a} | 62 ± 11^{b} | $66 \pm 10^{\circ}$ | < 0.001 |
| BMI | 24 ± 3 | 26 ± 4^{a} | 25 ± 4^{b} | $27 \pm 4^{\circ}$ | < 0.001 |
| Waist-hip ratio | 0.86 ± 0.08 | $0.89\pm0.07^{\rm a}$ | $0.88\pm0.08^{\rm b}$ | $0.90 \pm 0.06^{\circ}$ | < 0.001 |

Table 1. Clinical characteristics of subjects stratified according to hypertensive categories

CVA, cerebrovascular accident; *BMI*, body mass index; *a*, p < 0.05 versus normotensive; *b*, p < 0.05 versus normotensive *c*, p < 0.05 versus normotensive

and 570 (31%) with sustained hypertension. Participant characteristics and their BP readings are presented in Tables 1 and 2, respectively.

Determinants of WCH

Patients with white coat hypertension were older (p<0.001), and had a higher body mass index (p<0.001), waist-hip ratio (p<0.001), office SBP (p<0.001), office DBP (p<0.001), and albumin clearance ratio (ACR) (p<0.001) than normotensive subjects. Multivariate analysis revealed that age [odds ratio (OR)=1.029, 95% confident interval (CI)=1.001-1.057, p=0.04], office SBP (OR=1.145, 95% CI=1.119-1.172, p<0.001), and office DBP (OR=1.080, 95% CI=1.047-1.114, p<0.001) predict WCH (Table 3). At the 1-year follow up, 2% (16/790) of subjects who were normotensive developed sustained hypertension, and 10% (47/460) of subjects with WCH developed sustained hypertension (p<0.001; Table 4).

Sustained White-coat Masked Normotension p-value **Hypertension** hypertension hypertension 790 Number of subject 567 448 26 SBP (mmHg) 123 ± 11 150 ± 20^{a} $153\pm14^{\rm b}$ $129 \pm 10^{\circ}$ < 0.001 DBP (mmHg) 77 ± 8 86 ± 11^{a} $90\pm10^{\rm b}$ 80 ± 7 < 0.001 77 ± 12 79 ± 13 77 ± 11 76 ± 13 0.189 HR T-chol (mg/dl; mean \pm SD) 204 ± 40 201 ± 40 209 ± 37 210 ± 38 0.01 TG (mg/dl; mean \pm SD) 115 ± 67 137 ± 80^{a} 121 ± 70 $162 \pm 89^{\circ}$ < 0.001 LDL (mg/dl; mean \pm SD) 130 ± 34 126 ± 34 134 ± 33 138 ± 31 0.003 53 ± 13^{a} HDL (mg/dl; mean \pm SD) 57 ± 14 57 ± 14 $48\pm8^{\circ}$ < 0.001 Glucose (mg/dl; mean \pm SD) 103 ± 30 113 ± 38^{a} 106 ± 31 119 ± 38 < 0.001 HbA1c 5.9 ± 1.0 6.2 ± 1.2^{a} 5.9 ± 1.0 $6.4 \pm 1.3^{\circ}$ < 0.001 Uric acid 5.7 ± 1.4 6.1 ± 1.5^{a} 5.8 ± 1.4 $6.4 \pm 1.4^{\circ}$ < 0.001 Creatinine (mg/dl; mean \pm SD) < 0.001 0.9 ± 0.2 $1.0\pm0.3^{\mathrm{a}}$ 0.9 ± 0.3 $1.1 \pm 0.5^{\circ}$ Microalbumin (U) 30.4 ± 28.8 0.005 37.8 ± 33.0^{a} 36.4 ± 31.0 38.8 ± 39.0 eGFR 76.4 ± 19.2 71.3 ± 20.9^{a} 78.2 ± 22.4 68.6 ± 26.6 < 0.001 < 0.001 ACR 17.8 ± 31.7 32.4 ± 52.6^{a} $25.0\pm43.7^{\text{b}}$ 19.4 ± 25.9

Table 2. Clinical characteristics of subjects stratified according to hypertensive categories

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; T-chol, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein-cholesterol; eGFR, estimated glomerular filtration rate; ACR, albumin clearance ratio; a, p < 0.05 versus normotensive; b, p < 0.05 versus normotensive; c, p < 0.05 versus normotensive

Table 3. Determinants of white coat hypertension: Multivariate analysis

| Variant | Odds ratio | 95% Confident interval | p-value |
|------------|------------|------------------------|---------|
| eGFR | 1.001 | 0.984-1.018 | 0.900 |
| LDL | 0.998 | 0.992-1.005 | 0.639 |
| TG | 1.002 | 0.998-1.005 | 0.309 |
| Creatine B | 1.367 | 0.344-5.435 | 0.657 |
| Office SBP | 1.145 | 1.119-1.172 | < 0.001 |
| BMI | 1.032 | 0.963-1.106 | 0.373 |
| Age | 1.029 | 1.001-1.057 | 0.04 |
| DM | 1.140 | 0.549-2.366 | 0.726 |
| Office DBP | 1.080 | 1.047-1.114 | < 0.001 |

eGFR, *estimated glomerular filtration rate; LDL, low-density lipoprotein-cholesterol TG, triglyceride; SBP, systolic blood pressure; BMI, body mass index; DM, diabetes mellitus; DBP, diastolic blood pressure.*

Table 4. Patients who have progressed to sustained hypertension within 1 year

| White-coat hypertension, n (%) | Normotensive, n (%) | Odds ratio | р |
|--------------------------------|---------------------|------------|---------|
| 47/460 (10.2)* | 16/790 (2.1)* | 6.62 | < 0.001 |

*Ratios (%) of patients who progressed to sustained hypertension (home $BP \ge 135/85$ mm Hg or use of antihypertensives)

Discussion

WCH is defined as an elevated BP occurring in patients only in a medical care setting, where there is a difference between office BP and home or ambulatory BP. In patients with known hypertension, a white coat effect (WCE) may lead to an overestimation of the severity of hypertension, and thereby an unnecessary drug prescription. Due to the possible complications associated with being prescribed unnecessary antihypertensive medications, it is important to recognize patients with WCH in the clinic. Lindback et al.¹¹ elucidated that the independent predictors of systolic WCE included mean ambulatory BP, age, smoking status, antihypertensive treatment, and family history of cardiovascular disease. Mansoor et al. reported that increasing age was associated with an increase in the level of the WCE¹². Dolan et al. reported that being an older adult, female, and a non-smoker increased the risk of WCH¹³. In a large cohort of patients referred for further evaluation for clinically elevated BP, age was the only independent predictor of WCH. Corroborating previous studies, where WCH was present in a more than 15% of patients, it was also found that nearly a third of patients with SBP between 140-159 mmHg had WCH^{14, 15}. In the current study, WCH was found to be present in 25% of the general population, and 49% of those subjects were diagnosed with clinical hypertension. The prevalence was higher than that observed in other studies.

Event-based studies have shown that the risk of future cardiovascular disease is reduced in patients with WCH than in those with elevated ambulatory BP levels, even after controlling for concomitant risk factors¹⁶⁻¹⁸. The risk of future cardiovascular events did not differ between clinically normotensive subjects and subjects with WCH, which was defined as an average daytime ambulatory SBP of <130 mmHg and DBP of <80 mmHg¹⁹. BP measured over a 24-hour period by an ambulatory recording is the best method for estimating an individual's cardiovascular risk. This has been established in a large number of prospective cohort studies, most of which have shown that office BP has negligible prospective value, if the 24-hour BP is known^{16, 20-25}. Only two prospective studies have compared ambulatory BP with self-BP monitoring (the Ohasama²² and PAMELA studies¹⁰). Both of these studies found that the two methods had similar predictive values for future cardiovascular events. In principle, one would expect that 24-hour ambulatory BP would provide a better prediction of risk, since there are important aspects of the circadian profile that influence BP, which would only be detected via ambulatory recordings, not self/home BP measurements. An area where ABPM is particularly useful and superior to self-BP monitoring is the evaluation of antihypertensive drug efficacy in clinical trials^{26–28}. In clinical practice, however, clinic and self-monitoring of BP are the preferred methods for clinical evaluation of responses to treatment, as performing multiple ABPM sessions in the same patient is impractical. Self-measured BP readings may be more reproducible than ambulatory BP readings, if they are taken under standardized conditions.

Conclusion

The findings of the present study emphasize the importance of using a home BP monitor for the initial evaluation of patients, particularly older patients, who are referred for assessments of elevated clinic BP. Our study also supports the recommendation of regular follow-ups of in individuals with WCH.

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Study of domestic violence amongst women who apply to primary healthcare institution

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Abstract

Aim: This study has been done to establish the view point of married women to domestic violence against women in the region of Bolu city where we provide services and to determine their solution suggestions.

Material and methods: Currently married women (sharing the same house with her partner) between the ages of 15 and 49 who have applied to the 3 family practice offices which participated in our study between October 2011-November 2012 have been included in our study on a volunteer basis. A total of 2443 women participated in the study of descriptive nature. Data obtained from this study has been evaluated with SPSS (Statistical Program for Social Sciences) 20.0 package.

Results: 24.64% of the participators stated that there is domestic violence directed to them. A significant connection (p: 0.000) has been found between living at home as an elementary family and being subjected to domestic violence and it is observed that 64.45% of those exposed to domestic violence are living with their spouses and children at home.

Conclusion: Institutional and individual measures such as making legal regulations on violence against woman and providing the cooperation of the media and civil society organizations must be regulated to complement each other. As the women apply to the healthcare institutions quite frequently for both themselves and their children, improving the knowledge, attitude and behaviour of those who serve them concerning domestic violence is essential. In this regard, increasing the awareness of medical staff and focusing on the issue in trainings of the healthcare personally before graduation can be suggested.

Key words: Awareness, medical staff, spouse violence.

Introduction

Violence is an important community health care problem which can be seen in every aspect of human life and is ever- increasing in the world. World Health Organization (WHO) defines violence as: "The intentional use of physical force or power, threatened or actual, against another person that either results in or has a high likelihood of resulting in injury, death or psychological harm of the exposed person" (1).

Domestic violence is defined as all types of acts of violence directed at one of the spouses to force, humiliate, punish, show power or discharge anger-stress within a group that defines itself as family (2). The most commonly seen type of violence is the domestic violence used by men against women and children (3, 4). Conception and definition of domestic violence is formed on the cultural values of the society and individuals. In many societies, violence against women is conceived as an acceptable behaviour and is considered as a mundane feature of marriage. The fact that there is no reliable and serious support system for women who has been exposed to violence (5) and insufficiencies of legal regulations for domestic violence contributes to the increase in violence (6).

Half of the women on earth are subject to violence of their spouses. In a retrospective cohort study conducted by telephone survey in the United States, about 14.7% reported intimate partner violence of any type in the previous 5 years, of whom 45.1% of abused women experienced more than 1 type of intimate partner violence (7).

They have stated in the studies done that violence can be decreased by 75% with definition and interference by the staff in the primary healthcare institutions (8). However, violence against women is not cared sufficiently by healthcare professionals and questions on violence are not

directed much to women during their visits to the healthcare institutions (9, 10). In some studies, it is stated that healthcare professionals are reluctant in this issue (11) and in other studies, it is stated that women exposed to violence are hesitant in seeking help (12). Healthcare personel working in healthcare centres and particularly midwives and nurses are the first recourse that violence victims contact first and they must undertake a significant role in defining domestic violence. It is observed that there is also an increase in the other health problems of women (13) and children who are exposed to violence (14). Doctors generally write out a prescription for these women who have consulted to them for physical and emotional health problems and send them back to the same setting (15). Importance of creating awareness in healthcare professionals on this issue is clear.

Increasing social awareness in the issue is important in decreasing violence in society. Findings obtained from studies researching how women, as the group which is most exposed to violence in society define violence and how they perceive violence shall be effective in developing social awareness in violence.

This study has been done to establish the view point of married women to domestic violence against women in the region of Bolu city where we provide services and to determine their solution suggestions.

Method

Settings

This survey was conducted in the Bolu province in 2011-2012. Bolu is a city with a population of approximately. Our city has changed over to family practice on October 16th, 2006 and is amongst the cities in which the practice is carried out systematically. Number of working women and average income is quite high in our city compared to Turkey. The dominant religion in Bolu is Islam. Although women are subjected to violence because of their traditional role, they generally do not express their complaints.

Participants

This study has been done in a family health centre which has 7 family physicians. Average number of women aged between 15 and 49 per physician is 1252. Currently married women (sharing the same house with her partner) between the ages of 15 and 49 who have applied to the 3 family practice offices which participated in our study between October 2011-November 2012 have been included in our study on a volunteer basis. A total of 2443 women participated in the study of descriptive nature. Questionnaire consisted of 25 questions.

The questionnaire consisted of the first section containing the demographic characteristics of the participant; second section consisting of question on violence exposure conditions at home, presence of domestic violence between parents before marriage, her condition in resorting to violence on her children, domestic violence condition between the parents of her spouse, her spouse's condition in resorting to violence on the children and presence of types of violence; and, the third section consisting of open ended questions on why there is domestic violence against women according to the participants, reactions of the women and suggestions on what can be done.

All types of the physical, emotional, economic and sexual violence were included in violence in the study.

After the question 'Do you think there is violence against you at home?' was asked to the participant and an answer was taken more detailed questions on types of violence were directed. Violence awareness was determined by the answers given to detailed questions on types of violence by participants who gave the answer that there is no violence at home in the beginning.

The ones with similar characteristics in the answers given to open ended questions by the participants were later classified.

Procedure

After the ethical approval necessary or the study was obtained, the questionnaires were completed by the family physician serving in the office during a face to face interview with the participants. As soon as the consent of the women are taken the guarantee that the information given by them and the things they say will not be entered into the medical records and the interview terms were fulfilled in accordance with the confidentiality principle. A suitable environment for the quality of the study and to enable the women to express their feelings in difficult questions easily was provided. Participants who did not want to complete answering questions in the questionnaire were excluded from the study. The questions were asked again to the participants whose answer was "I do not know" to open ended questions and the response time was extended. Those who gave the same answer again were excluded from the study.

Women who wanted to come to the interview with their spouses were not included in the study. This is because, this condition might have had influenced the view of women and prevent them from revealing their emotions.

After the questionnaire was completed, the participants were informed on the domestic violence concept and what they can do in the present condition. The interview was completed in approximately 30 minutes.

Statistical analyses

Data obtained from this study has been evaluated with SPSS (Statistical Program for Social Sciences) 20.0 package. Frequency and percentage distribution of the data are given. Relations between variables are analysed with the chi-square test. When analysing difference between groups 0.05 is used as significance level and if it is p<0.05, it is stated that there is significant difference between the groups and if it is p>0.05, it is stated that there is no significant difference between the groups Forward Stepwise (Conditional) method is used in creating logistic regression model. Significance was completed in 8 steps.

Results

2443 women applying to our centre were included in the study. Certain socio-demographical data of the participants are summarized in table 1. 24.64% of the participators stated that there is violence directed to them at home. 71.76% of the participants live as an elementary family. A significant connection (p: 0.000) has been found between living at home as an elementary family and being subjected to domestic violence and it is observed that 64.45% of those exposed to domestic violence are living with their spouses and children at home. It is observed that 75.08% of those exposed to violence at home have lived in the city within the last five years and 48.17% got married with an arranged marriage with the consent of their families. It is seen that there is a statistically significant connection between their working conditions and there being subject to violence at home (p: 0.000). It is seen that 57.48% of those who are exposed to violence at home do not work and 86.38% have a regular income in the family (table 1).

55.32% of those subjected to violence at home expressed that they were also exposed to violence during pregnancy. It is seen that there is a statistically significant connection between being exposed to violence during pregnancy and there being subjected to violence at home (p: 0.000). 47.9% the participants described violence as physical. 56.31% of women who are exposed to violence at home stated that they have witnessed violence in their families as a child and 65.61% stated that they thought that their spouses were witness to violence in their families as a child. It is seen that there is a statistically significant connection between being witness to violence in the family as a child and there being subject to violence at home (p: 0.000). The spouses' being witness to violence in their families as a child increases their tendency to use violence 3.606 times (table 2).

It is found that 65.06% of the participants who are not subjected to violence at home are exposed to emotional-verbal violence. The response given by those subjected and not subjected to violence at home to violence types are shown (table 3).

They responded to the question 'Why do you think there is domestic violence against women?' by stating that there is no communication between the spouses with a high percentage (26.62%). It is seen that they answered by saying economic reasons with a high percentage (35.61%) to the question 'Why don't the women react when they are subjected to violence'.

The answers given to the question "What do you think your suggestions would be for the women not to be exposed to violence at home" with a high percentage were work for women under the government monitoring and that the woman should not think how she would live financially after parting (21.61%). Breakdown of the reasons causing violence are given on table 4.

| | | Violence | | | | | | | | |
|------------------|--|-----------|--------|------------|-------------|------------------|--|--|--|--|
| | | Y | es | N | 0 | р | | | | |
| | | n | % | n | % | _ | | | | |
| | Below 20 | - | - | 10 | 0,54 | | | | | |
| Age distribution | 20-29 | 109 | 18,11 | 515 | 27,97 | 0.000 | | | | |
| | 30-39 | 290 | 48,17 | 798 | 43,35 | 0.000 | | | | |
| | 40-49 | 203 | 33,72 | 518 | 28,14 | | | | | |
| | Literate | 4 | 0,66 | 103 | 5,59 | | | | | |
| Education | Primary school | 424 | 70,43 | 1231 | 66,87 | 0.000 | | | | |
| Education | High school | 132 | 21,93 | 299 | 16,24 | 0.000 | | | | |
| | University | 42 | 6,98 | 208 | 11,30 | | | | | |
| | Literate | 1 | 0,17 | 35 | 1,91 | | | | | |
| Education | Primary school | 391 | 64,95 | 832 | 45,19 | 0.000 | | | | |
| of spouse | High school | 156 | 25,91 | 568 | 30,85 | 0.000 | | | | |
| 1 | University | 54 | 8,97 | 406 | 22,05 | | | | | |
| 0. 10. 1 | Married | 599 | 99,50 | 1838 | 99,84 | 0.164 | | | | |
| Civil Status | Living Together | 3 | 0,50 | 3 | 0,16 | 0,164 | | | | |
| | 18 and below | 143 | 23,75 | 464 | 25,20 | 0.055 | | | | |
| Marriage age | 19 and above | 459 | 76,25 | 1377 | 74,80 | 0.255 | | | | |
| | With Family Consent, Arranged Marriage | 290 | 48,17 | 812 | 44,10 | | | | | |
| Marriage | With Family Consent, By Being Introduced and Getting | 241 | 40,03 | 910 | 49,43 | | | | | |
| manner | Without Family Consent, By Being Introduced and Get- | 20 | 3 33 | 85 | 4 62 | 0.000 | | | | |
| | ting On Well Without Family Consent by Eloping | 51 | 8.47 | 34 | 1.85 | | | | | |
| | 0-1 | 19 | 3 16 | 141 | 7.66 | | | | | |
| | 1-3 | 0 | 0.00 | 229 | 12 44 | | | | | |
| Vears of | 3-5 | 33 | 5.48 | 125 | 6 79 | 0.000 | | | | |
| marriage | 5-10 | 1/1/ | 23.92 | 276 | 1/ 99 | | | | | |
| marnage | 10-15 | 71 | 11 79 | 270 | 1/ 0/ | | | | | |
| | 15 and above | 335 | 55.65 | 795 | 43 18 | | | | | |
| | Provides a Regular Income | 221 | 36 71 | 347 | 18.85 | | | | | |
| | With Irregular Income | 35 | 5.81 | | 10,05 | | | | | |
| Employment | Retired | | 5,01 | 3/ | 1.85 | 0.000 | | | | |
| | No | 3/16 | 57.48 | 1/60 | 79.30 | | | | | |
| | Regular Income | 520 | 86.38 | 17/8 | 0/ 05 | | | | | |
| Employment | Irragular Income | 520 | 8.64 | 1/40 Q | 0.42 | | | | | |
| of spouse | Retired | 17 | 2.82 | 51 | 2 77 | 0.000 | | | | |
| of spouse | Not Employed | 17 | 2,02 | 24 | 2,77 | | | | | |
| Place Lived | Village | 82 | 12,10 | 162 | 8.80 | | | | | |
| in the Lost Five | Town | 68 | 11 20 | 02 | <u>0,00</u> | 0.000 | | | | |
| Voora | City | 452 | 75.08 | 95 1586 | 96.15 | 0.000 | | | | |
| | Spouse Children | 200 | 64.45 | 1226 | 67.14 | | | | | |
| | Spouse Children Mother in Law | 68 | 11 20 | 102 | 5 5 4 | | | | | |
| Permanently | Spouse Children, Mother in Law | 68 | 11,30 | 210 | 11 /1 | | | | | |
| Living in the | Spouse Children, Would III Law, Fauler III Law | 24 | 5.65 | 05 | 5 16 | 0.000 | | | | |
| House | Spouse Snouse Mother in Low | <u> </u> | 3,03 | 95 | 3,10 | | | | | |
| | All and Algo Others | 27 | 2,02 | 120 | 3,09 | | | | | |
| | All and Also Others | 2/ | 4,49 | 1219 | /,00 | | | | | |
| Monthly | May Income is More then may Expenditure | 121 | 04,93 | 1210 | 17.00 | 0.496 | | | | |
| Income | Ny Income is loss than my Expenditure | 121 | 20,10 | 331 | 17,98 | <u>,98</u> 0.486 | | | | |
| | IVIY Income is Less than my Expenditure | <u>90</u> | 14,95 | 292 | 13,80 | | | | | |
| Existence of | ICS No | 334 | 00,/0 | 1033 | 0.67 | | | | | |
| Children | Dragnant | 08 | 0.00 | 20 | 7,07 | 0.004 | | | | |
| | 1 ICZHAIIL | U | 1 0.00 | JU JU | 1.00 | | | | | |

Table 1. Breakdown of Certain Socio-demographical Characteristics of Women Exposed and not Exposed to Violence at Home (N: 2443)

| Variables in the Equation | | | | | | | | |
|---------------------------|---|---------------------------------|----------|-------|-------------------|----------------------|-------|--|
| | | B Df (Regression (Degrees of | | р | OR (Risk Coef- | 95.0% C.I. for OR | | |
| | | Coefficient) | Freedom) | | ficient) | High | Low | |
| | Age (constant) | 0,204 | 1 | 0,006 | 1,226 | 1,060 | 1,418 | |
| Step 8(h) | Spouse Education (1=High school and above) | 1,054 | 1 | 0,000 | 2,870 | 2,031 | 4,055 | |
| | Monthly Income (1=As Income increases) | 0,507 | 1 | 0,000 | 1,660 | 1,262 | 2,182 | |
| | Marriage age (1=Marriage age above) | 0,461 | 1 | 0,000 | 1,585 | 1,245 | 2,019 | |
| | Place lived in the last five years (1=living in city) | 0,509 | 1 | 0,000 | 1,664 | 1,428 | 1,939 | |
| | Employment of Spouse (1= with regular employment) | 0,678 | 1 | 0,000 | 1,971 | 1,395 | 2,783 | |
| | Spouse witnessing violence (1=witnessed violence) | 1,283 | 1 | 0,000 | 3,606 | 2,909 | 4,470 | |
| | Her witnessing violence (1=wit- nessed violence) | 0,601 | 1 | 0,000 | 1,825 | 1,474 | 2,258 | |
| | Constant | -2,388 | 1 | 0,000 | 0,092 | | | |

Table 2. Breakdown of Certain Socio-demographical Characteristics of Women Exposed and not Exposed to Violence at Home (N: 2443)

Table 3. Breakdown of Characteristics of Women Exposed and not Exposed to Violence at Home

| | | Those say th no viol ho | e who nere is ence at me | Those who say there is violence at home | |
|---------------|---|----------------------------------|-----------------------------------|--|-------|
| | | n | % | n | % |
| | Did he ever push you? | 51 | 39,2 | 286 | 39,1 |
| Dhygiaal | Did he ever throw something at you? | 32 | 24,6 | 235 | 32,1 |
| Physical | Did your spouse ever bit you when angry? | 16 | 12,3 | 0 | 0,0 |
| | Did he ever slap you? | 31 | 23,8 | 210 | 28,7 |
| | Does your spouse mostly approve what you do? | 130 | 14,2 | 319 | 13,5 |
| | Do you think he does not give attention? | 222 | 24,2 | 437 | 18,5 |
| | Do you think he shows sufficient love? | 203 | 22,2 | 391 | 16,6 |
| Physiological | Does he insult you? | 102 | 11,1 | 378 | 16,0 |
| - Verbal | Does he mostly criticize you negatively? | 96 | 10,5 | 336 | 14,3 |
| | Does he mostly look down on you? | 54 | 5,9 | 267 | 11,3 |
| | Does he usually ridicule you? | 56 | 6,1 | 143 | 6,1 |
| | Does he usually humiliate you? | 53 | 5,8 | 85 | 3,6 |
| г · | Do you have a say in the family budget? | 318 | 93,3 | 303 | 78,3 |
| Economic | Does your spouse take the money you earn? | 23 | 6,7 | 84 | 21,7 |
| Sexual | Does he force you to sexual intercourse when you do not want? | 21 | 100 | 17 | 100,0 |

* Column percentage

| | | n | % |
|-----------------------------|--|------|--------|
| | Economic problems | 544 | 22,27 |
| | Lack of communication between spouses | 650 | 26,61 |
| | Lack o respect to women | 32 | 1,31 |
| | Looking down on women | 238 | 9,74 |
| | The woman's unnecessarily being a busybody | 208 | 8,51 |
| Why do you | Bad habits o man like alcohol | 135 | 5,53 |
| think there is | The man's having suppressed psychological problems | 112 | 4,58 |
| domestic | Interference of the man's family | 102 | 4,18 |
| violence | Reflecting the behaviour of the surroundings on home | 61 | 2,50 |
| against women? | Because the men are raised dominantly | 161 | 6,59 |
| women: | Another relationship | 68 | 2,78 |
| | Because the woman does not earn money | 13 | 0,53 |
| | Lack of self-confidence of man | 102 | 4,18 |
| | When the woman does not humour | 17 | 0,70 |
| | Total | 2443 | 100,00 |
| | Economic weakness | 870 | 35,61 |
| | Fear of not being able to return to the family home | 443 | 18,13 |
| | Not to unsettle the order for the sake o the children | 362 | 14,82 |
| Why don't the | She must be keeping quiet because she is guilty | 85 | 3,48 |
| women react | Women are raised too passive to react. | 249 | 10,19 |
| when they are | She must have kept quiet when the result did not change when she reacted | 73 | 2,99 |
| subject to | She does not want to look unhappy to the surroundings. | 17 | 0,70 |
| violence? | Because they fear the results of reacting | 306 | 12,53 |
| | The families must be putting pressure against divorce | 21 | 0,86 |
| | She must be thinking that he will improve | 17 | 0,70 |
| | Total | 2443 | 100,00 |
| | The level of education of women must be raised | 90 | 3,68 |
| | The woman should not think how she will live after divorce with the | 520 | 21.61 |
| | employment of woman under government monitoring | 520 | 21,01 |
| What do you | The woman must be made to feel independent with supporting the socializing of woman. | 153 | 6,26 |
| think your | The partners should be given domestic communication trainings before marriage | 314 | 12,85 |
| suggestions would be for | Support of psychological peacemaker by the government for families with problems | 233 | 9,54 |
| the women not | They will not change, that's life, it's inevitable. | 292 | 11,95 |
| to be exposed | I do not have sufficient knowledge | 187 | 7,65 |
| to violence at | Number of safe houses supported by the government must be increased | 119 | 4,87 |
| home? | They must raise the men without gender discrimination as they are growing up. | 34 | 1,39 |
| | Employment opportunities to families with economic problems so that there will not be deteriorations | | 8,35 |
| | The girls and boys must be educated well starting from childhood | 289 | 11,83 |
| | Total | 2443 | 100,00 |

Table 4. Cause Breakdown of Violence

Discussion

The family physicians are responsible for following up the health of people registered in their lists. Health is not only physical well-being but also psychological well-being. Violence is an important health problem as it causes physical and psychological injuries. Family physicians should occasionally ask if the people registered in their lists are exposed to violence. This study is significant for family physicians with regard to the necessity of asking about domestic violence and explaining the method of asking this to people.

Violence directed at women is a global fact widespread in the world in all cultures and societies. Violence against women is observed in both domains within and outside of the family. Particularly men are indicated as resorting to domestic violence (16). The high rates of domestic violence in developing countries are recognized as global public health problems (17). In Korea 38% of wives reported being physically abused by their spouse, based on a survey of a random sample of women. In New Zealand 20% of 314 women surveyed reported being hit or physically abused by a male partner (18). In a study done a Mumbai, the proportion of domestic violence was found to be 36.9% (19). It has been found that 40.7% of women were subject to violence by their spouses in the study done by Güler et al. in Sivas (16), 27.7% in the studies done by Elmali et al. (20) and 26.1% in the study done by Nacar et al (15). Violence during pregnancy basically occurs as verbal and psychological. Physical or sexual violence is observed at a very low percentage (21). Most of the women who were subjected to violence were also exposed to violence during their pregnancy in our study as it is in literature.

Studies done show that the party most affected from violence which is considered as a public health problem is the children. This is because domestic violence leads to physical violence in the child besides causing harm on cognitive, behavioural, social and emotional functions and it is reported that abuse in the family harms more people than the sum of all genetic diseases (22). The manner of the child's being affected of the violence or how it will react to violence depends on age and his/ her cognitive development (23). Human psychology begins to take shape by taking the parents as a model in the family surroundings and the acceptance of the child of him/herself as an individual depends on the nature o the relationships established with family members and primarily with the mother. It is possible that the children who were subject to domestic violence will also become a violence user in the future. Furthermore, it is found that the violence witnessed or suffered as a child increases the potential of using violence in men and the potential of being exposed to violence in women (15, 24). The woman's and her spouse's witnessing violence in their families as a child increases their tendency to resort to violence 3.606 times for each of them. It is only possible for our children who will be the grown-ups of the future to be health adults and bring up healthy generations by the parents' avoiding violence and display positive attitudes and behaviours.

Among the protective factors identified in developing countries are higher socioeconomic status, women's economic independence, quality of marital relationship.(25) and higher levels of education among women (26). Elmali et al. have concluded that lack of money will lead to beating with 68.1% of the participants at their study (20). Efe et al. assessed that illiterate women is exposed to maximum moderate levels of violence (45.5%) and women graduated from high school and higher education are exposed to low of violence (68%) in their study (27). In this study, it was found that women who are illiterate, whose spouses are not employed and those in bad economic conditions are exposed to more violence. Economic problems can also cause not to be able to react. For the woman to be able to react and be able to stand behind the decision she makes her socio-economic level must be upgraded and legal regulations that will ensure that she will not have concerns for her future must be made. In other respects, it is seen that all of the women who stated that their spouses bite them when they are angry are illiterate women. It is seen that women who state that they are humiliated by their spouses (15.89%) are university graduates. As a matter of fact, it seems that violence against women in every segment not depending on socio-cultural level and only the type of the violence used is changing.

Violence is classified as verbal, emotional, economic and physical and it is stated that women who are subject to physical violence have been exposed to other types of violence before (20, 28). The women's conceiving violence as only physical cause them not to be aware even if they are subject to other types of violence and not to take steps in the direction of resolving violence (19). In our study, it was found that 29.06% of the participants who expressed that they have not been subjected to violence at home have been exposed to at least one the violence types. Various projects are carried out by relevant departments to reach large masses in our country. It is seen that awareness of women in domestic violence which is a social problem is not generally sufficient and it is necessary to continue these projects more intensively.

A healthy relationship does not mean a relationship in which there are no conflicts. In how healthy a manner the problems arising in those relationships are solved is important. To correct the individual's solving his/her problems by resorting to violence it must be ensured that the individual first acknowledges him/herself as an individual in the social life an develops empathy. Furthermore, the person must improve him/herself in ability to resolve conflicts and communication, and seek help by consulting to specialists (19). In addition risks that can generate violence and prevent communication such as unplanned pregnancies in prenatal and postnatal periods, births out of wedlock, young parents, psychological health of parents and handicapped people in the family can be determined as providing healthcare service and high risk families can be supported . Participants in our study have expressed that domestic violence results from the spouses' not being in communication with each other with a high percentage (26.61%) and 12.85% of the participants have suggested the provision of training on communication in family to couples before marriage. Arranged marriage is still a preferred marriage model culturally in our country. The woman and man who do not at all know each other are expected to share a life without the opportunity to get to know each other. Violence against women in those who marry by arranged marriages supporting the above is observed more. Premarital psychosocial support programs for individuals who will start a family can be developed in cooperation with social services professionals and psychologists. This can provide benefit in the individuals' overcoming communication problems in their marriage and being able to understand each other.

Those which are remarkable in the recommendations of the Committee of Ministers of the Council or Europe are on the subjects of bestowing human rights and fundamental freedoms on the women, guaranteeing their use, benefiting from them and protecting these rights. In the said recommendations the fact that the violence by men used against women is a basic structural and social problem arising from unequal power relations between women and men was underlined and the participation of men in actions that aims fighting against violence used against women (29). In a study done in India, it was stated that this widespread problem can not be explained by ignoring the isolation of women from the society. It is necessary to create opportunities for economic independence, necessary education and awareness beside strong legal support, establish alternative shelters and ensure a change starting from men and women and reflecting on the attitude and mentality of the society, legislation, execution and jurisdiction. The requirement of this day is to emphasize the egalitarian values in power and role relations of the society (30). In our study, suggestions of the participants for the women's not being subject to violence at home were to provide employment opportunity to women under government monitoring with a high percentage. Her being able to react would mean moving away from the surroundings and that would mean taking many responsibilities. Employment of women can be expressed as her feeling economically strong.

The 'Do you think there is violence against you at home?' question was asked for the current days and the past was not probed. If we were able to compare the answers given by women and answers at the separate interviews done only with their spouses, we could have revealed the missing pieces in the domestic relationships arising from the differences in the perception of behaviours between the spouses. The policlinic is busy because of the size of population per family physician. The entire 15 to 49 age women population could not be reached or such reasons. If it were possible to reach them, our results could have been stronger. We could not come across such a research done in the family health centre after it was changed to family practice. We consider that family physicians are the most suitable physician group in determining the risk map in our society and that our study can be an encouragement to studies that will be done on the concept of violence against women in the first step.

Conclusion

Although domestic violence against women is a frequent problem in the society, it may not be easily diagnosed like common chronic illnesses. Even if it is diagnosed, every woman may not express this situation easily. It must be ensured that family physicians and family healthcare personnel get the training necessary or identifying and treating domestic violence.

The women should be informed on the domestic violence and its types, their level of education must be improved and it must be enabled that they gain their economic independence. Institutional and individual measures such as making legal regulations on violence against woman and providing the cooperation of the media and civil society organizations must be regulated to complement each other. As the women apply to the healthcare institutions quite frequently for both themselves and their children, improving the knowledge, attitude and behaviour of those who serve them concerning domestic violence is essential. In this regard, increasing the awareness of medical staff and focusing on the issue in trainings of the healthcare personnel before graduation can be suggested.

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Epithelioid sarcoma of scrotal sac: a rare entity

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Abstract

A 54 years old male presented with a pathological fracture of right proximal femur and painless left scrotal mass whereby biopsy denoted as a metastatic epitheloid sarcoma. The scrotal mass remained dormant with size of 3 cm for the last eight years, which progressively increasing in size within a period of four months prior to fracture.

Local evaluation of proximal femur revealed lytic lesion with soft tissue component involving right proximal shaft of femur and CT scan showed large lobulated soft tissue mass within left scrotal sac. Furthermore, multiple lung nodules in both lung fields were observed.

The osteolytic lesions of the proximal femur with soft tissue involvement were resected marginally and reconstruct with proximal femur replacement. The scrotal masses were resected combined with left orchidectomy. Both operative field received 60 k Gy external beam radiotherapy and after a year, patient walking unaided with no local recurrence.

Current case highlighted rare presentation of epitheloid sarcoma with scrotal mass and remained dormant for long period and spontaneous aggressive with bone and pulmonary metastases.

Key words: scrotal sac tumour; epithelioid sarcoma; bone and pulmonary metastases

Introduction

Epithelioid Sarcoma (ES) is a rare condition, growing slow with painless pattern.¹ The ES are not easy to diagnose and can be puzzled with assortment of malignant and benign conditions;

particularly granulomatous process, synovial sarcoma and ulcerating squamous cell carcinoma.²

It is frequently observed in adults with deep seated and occurs in perineum, sex organs, pelvis and thighs.² It is more aggressive with poorer prognosis with tendency for local recurrence and early metastases. The proximal type epitheloid sarcoma are uncommon undifferentiated soft tissue sarcomas usually seen in adults with epithelioid features and a frequent rhabdoid phenotype on histopathology³ thus posing diagnostic intricacy and has to be differentiated from extra-renal rhabdoid tumour, synovial sarcoma, angiosarcoma and melanoma.^{3,4}

The most common primary site for epithelioid sarcoma is distal upper extremities although cases of involvement of other parts of the body like vulva, penis and spine have been reported.^{1,5}

Case report



Figure 1. Before operation



Figure 2. Intra-operatively, after excision of tumour. Both testes are preserved. Margin is clear



Figure 3. Excised tumour, measures 525 g in weight



Figure 4. After operation

Male of 54 years age presented with pathological fracture of subtrochanteric right femur after right side fall (Figure 6). There was presence of scrotal swelling which was painless and claimed to be small and dormant for eight years and progressive increased in size for past 4 months. There was no associated loss of appetite, loss of weight and denied history of recurrent fever.



Figure 6. Pathological fracture of right proximal femur

Examination revealed large left scrotal mass measuring 10cm x 8cm in size which was hard in consistency. It was non-tender; testes were normal and separated from the scrotal mass, while no lymph nodes felt.

Based line investigation was unremarkable with Hemoglobin of 12g/l and tumor markers of Serum PSA, CEA, CA 125, Alpha Fetoprotein, Beta-HCG were all negative. Ct scan (Figure 7) staging revealed large lobulated soft tissue mass within left scrotal sac measured with size of 10.0 x 7.5 x 8.9 cm. Multiple lung nodules in both lung fields were found. The pathological fracture was associated with lytic lesion with soft tissue component involving right proximal shaft of femur was noted. Multiple small lymph nodes were found in bilateral supraclavicular fossa and bilateral axilla. Bone Scan showed increased uptake at proximal femur consistent with solitary bone metastasis. Biopsy of the proximal femur bone suggests metastatic sarcoma.

Patient underwent resection of the proximal femur and reconstruction with proximal femur replacement and subsequently excision of left scrotal tumour.

Histopathology evaluation of both specimen showed that similar tumor morphology which composed of epithelioid cells exhibiting large pleomorphic nuclei with some of the tumor cells



Figure 7. CT of the scrotal sac tumour

exhibit rhabdoid differentiation. Pseudoangiosarcomatous changes and lymphovascular invasion are seen in many areas. Immunohistochemical staining was positive for epithelial membrane antigen (EMA), vimentin, and cytokeratin (CK); but negative for CD34, and S100.

Both operative field received 60 k Gy external beam radiotherapy and currently after a year, patient able to walk unaided with no local recurrence

Discussion

Epithelioid Sarcoma (ES) founds rare, with male to female ratio 3:1.⁶ ES occurs mainly in the distal extremities as a subcutaneous or deep dermal mass. The tumour is slow growing and frequently affects the subcutaneous tissues, fascia, or tendon sheaths in the extremities.⁷ Due to its painless and indolent growth; the tumour has been present for long duration prior to presentation.⁸ Epithelioid Sarcoma would have has been advanced by the time of presentation, which reflecting with current case.

Literature revealed distinct clinical profile whereby proximal-type ES is somewhat more aggressive and metastasizes earlier than conventional Epithelioid Sarcoma.⁹ Right diagnosis of proximal type epithelioid sarcoma is important because it can easily be erroneous for other malignant tumours that exhibit epithelioid morphology. Histologically, a conventional ES shows a nodular arrangement made of epithelioid cells and spindle cells, with central necrosis. Whereas, Proximal-type Epithelioid Sarcoma is characterized by predominantly large-cell, epithelioid cytomorphology, with rhabdoid features and lack of granuloma-like pattern. ^{7,9}

Both conventional Epithelioid Sarcoma and proximal-type Epithelioid Sarcoma retains the same immunohistochemical profile. Most important immunohistochemical markers are vimentin (100%), epithelial membrane antigen [EMA] (92.5%), cytokeratin [CK] (75%), CD34 (62.5%) and S100 (10%). Desmin is found in 57% of proximal-type Epithelioid Sarcoma cases but is not found in conventional Epithelioid Sarcoma.^{7,10}

As for present patient, immuno-histochemical profile was positive for Vimentin, EMA and CK; negative for CD34 and S100.

Currently, another characteristic feature found in epithelioid sarcomas, both conventional and proximal-type, is a loss of nuclear INI1 expression. Inactivation of SMARCBI/INI1 tumour suppressor gene at Chromosome 22 has been identified as a specific genetic marker of malignant rhabdoid tumours. Recent study showed loss of INI1 expression has been reported in about 81% of Epithelioid Sarcoma cases.¹¹

This immunohistochemical profile is useful as diagnostic markers but has no prognostic impact.¹⁰

Prognosis

Literature shows high rate of recurrence (more than 55%), which can be reduced to less than 40 % in patients who underwent wide local excision.^{6-8, 12-14}

However, local recurrence rate for proximaltype Epithelioid Sarcoma after marginal resection was 83.3%.⁷ Amputations as primary treatment or recurrence treatment does not improve the survival advantage.¹³ Therefore, wide local excision with negative margin is the main stay of treatment. Marginal excision is inadequate due to the high rate of local recurrence.¹⁴ Duration between excision and recurrence ranges from 1 month to 18.8 years.^{6,12} Most occur within 1 - 2 years after resection and median time to recurrence after resection is 1.23 years.^{6,14}

Rekhi and colleagues reported that 7 year disease free survival in conventional Epithelioid Sarcoma was 19.4% while proximal type is nil. Overall survival rate was 90.2% in conventional type as compared to 31.3% in proximal type Epithelioid Sarcoma.⁷ Unfavourable parameters include deeper location, tumour size (>5cm), higher tumour stage, early metastasis, and proximal location of tumour.^{7, 12}

Treatment

Recommended treatment of Epithelioid Sarcoma has mainly been deduced from treatment of soft tissue sarcoma. Surgical excision with clear margin, preferably wide excision, is the mainstay of treatment. Chemotherapy and radiotherapy for Epithelioid Sarcoma are still controversial.

Chemotherapy regimens mainly consist of doxorubicin with or without combination with ifosfamide; and combination of taxol, taxotere and carboplatin.⁶ Chemotherapy for non-metastatic Epithelioid Sarcoma does not seem to provide any benefit because patients who underwent chemotherapy have a recurrence rate of 50% as compared to 55% rate of recurrence in patients who don't have.⁶ Additional studies are needed to support the use of chemotherapy for non-metastatic Epithelioid Sarcoma.

On the other hand, chemotherapy for metastatic Epithelioid Sarcoma appears to confer a partial response in 15% and stable disease in 60% of the patients treated. However, due to the aggressive nature of the disease, responses to chemotherapy are only of short duration. Median overall survival is 4.2 years after commencing palliative chemotherapy.¹⁵ Adjuvant radiotherapy has proven beneficial in management of soft tissue sarcoma. Studies on soft tissue sarcoma by Kinsella and Talbert noted better outcome in patients who underwent adjuvant radiotherapy after primary surgery as well as after surgery for local recurrence.^{16, 17} However, there are limited data in Epithelioid Sarcoma to support its benefit due to small sample size and limited duration of follow-up. Frequently radiotherapy were given for marginal resection, wide local excision, locoregional recurrence or palliative radiotherapy, although its benefit has not clearly been described.^{14, 16-18}

A study showed no significant advantage for adjuvant radiotherapy; local recurrence rate was similar in patients who underwent adjuvant radiotherapy versus surgery alone.¹³ Another study on Epithelioid Sarcoma noted a low local recurrence rate when Epithelioid Sarcoma patients were treated with adjuvant therapy.¹⁸

Combined chemo-radiotherapy with surgery has been shown significantly reduced distant metastasis rate and increased overall survival rate in patients with soft tissue sarcoma.¹⁹ However, data is sparse on combination therapy for treatment of Epithelioid Sarcoma. Livi et al recommended a follow-up regime every 3 monthly, with clinical examination and CXR for the first 3 years; then 6 monthly for another 2 years; then annually.¹³ Extended follow-up is indicated as recurrence can occur after many years.

Conclusion

The diagnosis of proximal type Epithelioid Sarcoma for present case is based on histological finding of large, rhabdoid cells in a tumour with epithelioid morphology and exhibits immunohistochemical positivity for Vimentin, EMA and CK. Unfortunately, patient presented with stage IV disease (metastasis to lungs and bone). Literature has shown that Chemoradiotherapy has a role as an adjuvant to the surgery for metastatic Epithelioid Sarcoma, however the outcome might be unfavourable as the nature of this particular cancer is very aggressive.

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Frequency *Escherichia Coli* in the urine of outpatients home health Ilidza

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Abstract

The family Enterobacteriaceae includes a large group of pathogenic and conditionally pathogenic intestinal bacteria most of which normally inhabits the mucous membrane of the large intestine of humans and animals. Their name comes from the place of residence (intestine). The most common localization of infections caused by these bacteria is the genital tract, nasopharynx, lung, open wounds. Enterobacteriaceae have common morphological, culturally and antigenic properties. Cause similar infections, and are treated with the same group of antibiotics. In addition to strictly pathogenic (Salmonella, Shigella and Yersinia) other bacteria in this family (Escherichia coli, etc.) usually cause disease in two cases, when their frequency in the intestinal micro biota rises above 50 % and when they change the residence. Escherichia coli are one of the most commonly isolated organisms in medical microbiology lab. It was first identified by the German pediatrician Theodor Escherich during the study of intestinal micro biota in children. In this study, participated outpatients Ilidža Health Center in the period from 01.03.2012. to 01.09.2012. Year. On the occasion of the research methods were used in the primary and secondary processing of urine. The investigation determined distribution of respondents by sex, of which 6395, or (19.2%) males and 26,892 or (80.7 %) females, by which it can be concluded that women suffer more than men. These results coincide with reports from WHO, it is estimated that more than a billion women in the course of one year, Sirona world will suffer some form of urogenital infection that is transmitted through sexual contact. Sirona world will suffer some form of urogenital infection that is transmitted through sexual contact. During our survey, respondents also showed a different distribution in relation to the period (month), so she's in women was most

frequent in May (5145, 19.1%) and least common in August (3987 or 14.8%). For male respondents highest frequency was observed in April (1890 or (9.6%), and lowest in July and stood at 600 (9.4%). Statistical analysis of the material for the period from 01.03.2012. to 01.09.2012. year showed that regular findings were 26,854 or (80.7) than that of positive findings was 6406 or (19.3%). Analyzing the positive findings in the period (month), it is evident that the March month 2012th year was full with the highest number of positive findings or 1175 (18.3%), and the month of August 2012 had the lowest number of positive findings and 853 (13.4%) Isolation and identifications of the test material was determined number of species of bacteria. Of the total number of outpatients (n=33260) representation of bacterial species ranged E. coli 15.0%, Proteus mirabilis 1.2 % of cases, Enterobacter sp. 0.4%, Streptococcus haemolyticus gr. "B" 0.4%, Enterobacter aerogenes 0.5%, Proteus mirabilis in 1.2% of cases. Statistics, the processing of the test material was confirmed on the basis of research that regular findings were 26,854 or (80.7 %), while the positive findings of any 6406 or (19.3%) of the total number $n = 33260^{th}$

Prevention of infections caused by *E. coli* in outpatients of the Health Ilidža can recommend cranberry preparations and application of probiotics.

Key words: pattern, E. coli, urine, Health Ilidža.

Introduction

Escherichia coli is a Gram- negative, mobile, no spores, facultative anaerobic bacteria that live in the intestinal tract of humans and other warmblooded animals. It is very resistant (a capsule). Mainly lactose is positive, on endo agar produces purple colonies with a metallic sheen, and their metabolism very acidify surface. Selenium salts and bile salts inhibit the growth of *E. coli*. Natural reservoirs of enter pathogenic strains are the hoses of people (EPEC, ETEC, EIEC, EAEC) or domestic animals (ETEC, EHEC) (1). These microorganisms are transmitted by direct contact or through contaminated food and water. E. coli is a common cause of acute and chronic urinary tract infections can lead to acute or chronic cholecystitis (2). It is also known to cause neonatal meningitis, sepsis and abscesses in a number of organ systems, appendicitis, peritonitis, postoperative wounds, acute enteritis in humans and in animals, and generally causing "traveler's diarrhea", dysentery, hemorrhagic colitis, which is often called "bloody diarrhea" (3). Usually taking the material to demonstrate E. coli is urine, cerebrospinal fluid, blood, and the most common method for identifying E. coli is a urine culture, (urine samples and sending the microbiological examination). Contaminants in the urine due when urinating, but they are very much affected by frequent than the pathogens multiplied in infected bladder. Therefore, contaminants usually do not achieve concentration in urine greater than 103 CFU/ml (3, 4). Finding the correct number of bacteria in the urine, depends on sampling and transport of samples and includes washing vulva before sampling, taking MSU and fast delivery of samples to the microbiology laboratory or in the event of further transportation of the sample should be stored at $+4^{\circ}$ C for no longer than 24h. Distinguishing between contamination and true bacteriuria based on the number of bacteria in the urine should always be accompanied by clinical judgment, because the number of bacteria in the urine, emphasized, only the degree of probability that a certain number of bacteria means bacteriuria, and no contamination (5). The primary pathogens as we have already pointed out include Escherichia coli, and Staphylococcus saprophyticus (6, 7). Overall E. coli is the most common cause of uncomplicated and complicated urinary tract infections, and, as a dominant pathogen but occurs primarily in uncomplicated urinary tract infections in young women, where it causes at least 80% (8,9). Strains of E. coli, which easily cause urinary tract infections belong to a limited number of O serogroups (O1, O2, O4, O6, O7, A16, A18 and Ø75). The share of these serogroups in normal micro biota intestine is 28%, while their representation among agents pyelonephritis about 80% and about 60% of cystitis (10). Virulence factors that E. coli are the primary pathogen and allow her to the fecal tank invades the bladder and kidney, and are well studied and include adhesins that E. coli attached to the epithelial cells of the urinary tract (11). The most important virulence factors of E. coli adhesions that is located on top of fimbriae or on the surface of bacterial cells (12). Most adhesions are lections that bind to the oligosaccharide receptors on the surface of epithelial cells of the urinary tract. Staphylococcus saprophyticus distinguished among other staphylococci as Uropathogen because of its ability to grow in the urinary tract and successful adherence to uroepithelial cells (13). S. saprophyticus produce urease in larger quantities than other staphylococci, which leads to the formation of ammonia, which use the S. saprophyticus as a source of nitrogen, and which leads to mucosal damage and increased pH of urine (14). Further virulence mechanisms include the formation of biofilms (15).

The aim of this study was to determine the prevalence of *E. coli* from urine of outpatients of the Health Ilidža and how to achieve prevention of infections caused by *E. coli* in outpatients' home health Ilidža.

Materials and Methods

The survey respondents participated as outpatients of the Health Ilidža period of 01.03.2012. to 01.09.2012. Year. During the research methods were used in the primary and secondary processing of urine. Samples were taken in sterile containers with sterile lids. For microbiological analysis of urine taken midstream first morning urine. Immediately before sampling was necessary to wash the skin genitals with soap (the area around the urethra), rinsed with tap water and without deleting miss a few milliliters of the first jet out, and then midstream urine urinate in a sterile container. After processing the samples, analyzed their distribution in relation to gender and period of testing. Were entered in MS Excel 2007. Data after sorting, grouping and control transported to statistical software package SPSS 16.0, where after defining variables performed statistical analysis of the data. The results are presented in the appropriate number of tables and graphs.

Results

Tests were conducted in the period from 01.03.2012. to 01.09.2012. The presented results are in the table 1.



Figure 1. Distribution of respondents by sex for the period from 01.03.2012. to 01.09.2012.

Table 1 and chart 1. shows distribution of respondents by gender where clearly shows that the number of female respondents was 26 892 or (80.7%) and the number of male respondents was 6395, or (19.2%) of the total number of respondents 33287th Respondents also showed a different distribution in relation to the period (month) so that she stood up for women in the month of May 5145, or (19.1%) and the lowest in August 3987, or (14.8%). For male respondents largest distribution was recorded in April 1890, or (29.6%), and lowest in July and stood at 600 or (9.4%).

Statistical analysis of the material for the period from 01.03.2012. to 01.09.2012. year showed that regular findings were 26,854 or (80.7) than that of positive findings was 6406 or (19.3%). Analyzing

the positive findings in the period (month) to conclude that the month of March 2012th year was full with the highest number of positive findings or 1175 (18.3%), and the month of August 2012 had the lowest number of positive findings and 853 (13.4%) of the total $n = 33\ 260$ (Table 2, Figure 2).



Figure 2. Statistical analysis of materials for the period of 01.03.2012. to 01.09.2012.



Figure 3. Display of isolated bacteria from the test substances for the period from 01.03.2011. to 01.09.2012.

Table 1. Showing the distribution of respondents by sex for the period from 01.03.2012. to 01.09.2012.

| Month | Male | Female | Total |
|--------|--------------|---------------|--------------|
| March | 1520 (23.8%) | 4940 (18.4%) | 6460 (19.4%) |
| April | 1890 (29.6%) | 4144 (15.4%) | 6034 (18.1%) |
| May | 680 (10.6%) | 5145 (19.1%) | 5825 (17.5%) |
| June | 705 (11%) | 4394 (16.3%) | 5099 (15.3%) |
| July | 600 (9.4%) | 4282 (15.9%) | 4882 (14.7%) |
| August | 1000 (15.6%) | 3987 (14.8%) | 4987 (15%) |
| Total | 6395 (19.2%) | 26892 (80.7%) | 33287 |

Table 2. Showing statistical analysis of materials for the period of 01.03.2012. to 01.09.2012.

| Month | Neat | Positive | Total |
|--------|---------------|--------------|--------------|
| March | 5280 (19.7%) | 1175 (18.3%) | 6455 (19.4%) |
| April | 4865 (18.1%) | 1162 (18.1%) | 6027 (18.1%) |
| May | 4749 (17.7%) | 1071(16.7%) | 5820 (17.5%) |
| June | 4045 (15.1%) | 1050 (16.4%) | 5095 (15.3%) |
| July | 3792 (14.1%) | 1090 (17%) | 4882 (14.7%) |
| August | 4123 (15.4%) | 858 (13.4%) | 4981(15%) |
| Total | 26854 (80.7%) | 6406 (19.3%) | 33260 |

| Month | E. coli | Proteus mirabilis | Streptococcus haemolyticus "B" | Enterobacter aerogenes | Proteus species | Streptococcus faecalis | Enterobacter species |
|--------|---------|----------------------|-----------------------------------|---------------------------|--------------------|---------------------------|-------------------------|
| March | 930 | 52 | 37 | 28 | 22 | - | - |
| April | 907 | 73 | 49 | 22 | - | 26 | - |
| May | 830 | 82 | 40 | 46 | - | 35 | - |
| June | 844 | 73 | - | 26 | - | 29 | 32 |
| July | 881 | 65 | - | 33 | - | 23 | 45 |
| August | 673 | 63 | - | - | - | 19 | 68 |
| Total | 5065 | 408 | 126 | 155 | 22 | 132 | 145 |
| Total | (15%) | (1.2%) | (0.4%) | (0.5%) | (0.1%) | (0.4%) | (0.4%) |

Table 3. Display of isolated bacteria from the test material for the period from 01.03.2012. to 01.09.2012.

The total number of isolated bacteria for the period from 01.03.2012. - 01.09.2012. = 33260

Table 4. Displaying E. coli in absolute isolated from the test substances for the period from 01.03.2011. to 01.09.2012. the

| Period | March | April | May | June | July | August |
|---------|-------|-------|-------|------|------|--------|
| | 2012. | 2012. | 2012. | 2012 | 2012 | 2012 |
| E. coli | 930 | 907 | 830 | 844 | 881 | 673 |

Furthermore, the results of ambulatory patients of the Health Ilidža period of 01.03.2012. to 01.09.2012. Year showed abundance of species from isolates (Table 3, Figure 3). *E. coli* is represented in 15% of cases. *Proteus mirabilis* was present in 1.2% of cases, *Enterobacter sp.* 0.4%, *Streptococcus haemolyticus* gr. "B" 0.4%, *Enterobacter aerogenes* 0.5%, and *Proteus mirabilis* in 1.2% of cases and a number of other bacteria that are less common, but still important in certain situations.

From Table 4 it can be concluded that in March 2012 *E. coli* isolated 930 times in the examined material, a minimum number of times he was isolated in August 2012 673 times.



Figure 4. Display of isolated bacteria from the test substances for the period from 01.03.2012. to 01.09.2012. the

Urine analysis test material in ambulatory patients of the Health Ilidža during the semi-annual tests also confirmed the biggest representation *E*. *coli* Graph 4.

Discussion

In this study the period from 01.03.2012. until 01.09.2012. were included 33287 ambulatory patients of both sexes of the Health Ilidža, of which there were 6395 (19.2%) males and 26892 (80.7%) females, from which it can be concluded more incidence of women than males. These results coincide with reports from WHO, it is estimated that more than a billion women in the course of one year, Sirona world will suffer some form of urogenital infection that is transmitted through sexual contact. Causes urogenital infection members are physiological human micro biota. This fact often complicates interpretation of bacteriological findings because it is sometimes difficult to decide whether the isolated microorganism true pathogen or a saline contamination micro biota. During our tests, the subjects also showed a different distribution in relation to the period (month) so that she stood up for women in the month of May 5145, or (19.1%) and the lowest in August 3987, or (14.8%). For male respondents largest distribution was recorded in April 1890, or (29.6%), and lowest in July and stood at 600 or (9.4%) Table 1, Figure 1 This fact is difficult to confirm given period (month) affects the distribution of respondents, and we believe that in the coming period should extend the period of testing. Statistical analysis of the material for the period from 01.03.2012. to 01.09.2012. year showed that regular findings were 26,854 or (80.7) than that of positive findings was 6406 or (19.3%). Analyzing the positive findings in the period (month), it is evident that the March month 2012th year was full with the highest number of positive findings or 1175 (18.3%), and the month of August 2012 had the lowest number of positive findings and 853 (13.4%) of the total $n = 33\ 260$ (Table 2, Figure 2).

Results of the analysis of ambulatory patients of the Health Ilidža period of 01.03.2012. to 01.09.2012. year showed abundance of species from isolates (Table 3, Figure 3). E. coli is represented in 15.0% of cases. Proteus mirabilis was present in 1.2% of cases, Enterobacter sp.0,4%, Streptococcus haemolyticus gr. "B" 0.4%, Enterobacter aerogenes 0.5%, while Proteus mirabilis in 1.2% of cases and a number of other bacteria that are less common, but still important in certain situations. These data can be linked in the interpretation limits to the concentration of bacteria in the urine. Besides the concentration of bacteria in the urine to the interpretation of the results impact sample types, clinical, patient gender and the number of isolated bacterial strains. A large number of bacterial species typically means contamination of the urine. Semi- examination also confirmed the highest prevalence of type E. coli in urine or 5065 (15.0%) cases in ambulatory patients of the Health Ilidža. These results coincide with the data of several authors (4, 7, 10, 12, 15). Responding to another set goal in this paper on how to achieve prevention of infections caused by E. coli in outpatients of the Health Ilidža can recommend cranberry preparations. According to the literature in recent years in the United States, and in other parts of the world have been widely used cranberry preparations primarily to prevent recurrence of urinary infections. The mechanism of action of cranberry is blocking the binding of E. coli to epithelial cells of the urinary tract, the active substance proanthocyanidins containing only cranberry Vaccinium macrocarpon. Further application of probiotics is recommended for the prevention of urinary tract infections. Probiotics suppress the colonization of pathogenic bacteria, thus decreasing the risk of infection.

Conclusion

The survey was conducted from 01.03.2012. to 01.09.2012. year as participants attended outpatients of the Health Ilidža. It was included 33,287 patients of both sexes, of which there were 6395 (19.2%) males and 26892 (80.7 %) females, by which it can be concluded that women suffer more than men. These results coincide with reports from WHO, it is estimated that more than a billion women in the course of one year, Sirona world will suffer some form of urogenital infection that is transmitted through sexual contact. During our survey, respondents also showed a different distribution in relation to the period (month), so she's in women was most frequent in May (5145, 19.1%) and least common in August (3987 or 14.8%). For male respondents highest frequency was observed in April (1890 or (9.6%), and lowest in July and stood at 600 (9.4%). Statistical analysis of the material for the period from 01.03.2012. to 01.09.2012. year showed that regular findings were 26,854 or (80.7) than that of positive findings was 6406 or (19.3%). Analyzing the positive findings in the period (month), it is evident that the March month 2012th year was full with the highest number of positive findings or 1175 (18.3%), and the month of August 2012 had the lowest number of positive findings and 853 (13.4%). Results showed abundance of species from isolates, E. coli is represented in 15% of cases. Proteus mirabilis was present in 1.2% of cases, Enterobacter sp. 0,4%, Streptococcus haemolyticus gr. " B " 0.4%, Enterobacter aerogenes 0.5%, Proteus mirabilis, while the 1.2%. Semi- examination also confirmed the highest prevalence of type E. coli in urine or 5065 (15.0%) cases in ambulatory patients of the Health Ilidža. Prevention of infections caused by E. coli in outpatients of the Health Ilidža can recommend cranberry preparations and application of probiotics.

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Acute obscure gastrointestinal bleeding caused by lumbricoides infestation: A case report

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Abstract

We report a rare case of acute obscure gastrointestinal bleeding, after general upper endoscopy and colonoscopy examination without positive finding, caused by *Ascaris lumbricoides* diagnosed by capsule endoscopy and double balloon endoscopy. The *A. lumbricoides* was retracted by double balloon endoscopy. After antihelminth treatment, the patient showed no bleeding sign.

Key words: Obscure gastrointestinal bleeding, capsule endoscopy, double balloon endoscopy, *Ascaris lumbricoides*, endoscopy interventional therapy, antihelminth drug; acute gastrointestinal bleeding, small intestine computer tomography.

Introduction

Obscure gastrointestinal bleeding (OGIB) is a condition in which the bleeding site remains undiagnosed after routine examinations including upper gastrointestinal endoscopy and colonoscopy[1]. After a decade of improvements in techniques for determining the etiology of OGIB, many causes has been identified, e.g. angiodyplasia, smallbowel tumors, ulcers, and erosions [2]. However, in some cases, the causes of OGIB remain unidentified. We describe here a case of OGIB caused by a parasite, *Ascaris lumbricoides*, presenting as acute GI bleeding.

Case report

51-year-old man presented with a 2 year history of intermittent fatigue, 1 week of melena, and was found to be severely anemic. There was no history of nonsteroidal anti-inflammatory drug (NSAIDs) or anticoagulation drug intake. There was a history of HBV infection of 51 years without systemic antiviral treatment. There was also a history of alcohol intake, but he had been abstinent for half a year prior to admission. Physical examination revealed pale mucosa, but no other positive findings. The hemoglobin was 69 g/L (120-160 g/L), platelet count was within normal limits, and the eosinophil count was 0.08×10⁹/L $(0.02-0.5\times10^{9}/L)$. Stool was positive for occult blood, but negative for ova or adult parasitic organisms. Liver function and coagulation tests were all within normal limits. Upper endoscopy revealed chronic non-atrophic gastritis, and colonoscopy visualized the terminal ileum with no abnormalities. A small intestine computer tomography (CT) scan with two-phase enhancement and IV contrast showed no gastrointestinal abnormalities. However, there were signs of mild cirrhosis, but no signs of splenomegaly, ascites, or other evidence of portal hypertension on physical examination. A capsule endoscopy was performed, which passed the pylorus at 9 min and entered the large intestine at 4 h 2 min. A worm was found between 11 min 19 s to 23 min (Figure 1). After 27 min, the small intestine lumen became filled with dark red intestinal fluid (Figure 2). After the capsule examination, a double balloon endoscopy by the oral route was performed, and an A. lumbricoides was found in the proximal jejunum and was retrieved with biopsy forceps (Figures 3, 4). Double balloon endoscopy was advanced farther into the jejunum, but no other parasite, ulcer, or blood was found.

The patient was prescribed with albendazole, 0.4 g QD p.o for one week, and followed for 3 months without any further signs or symptoms of bleeding. The hemoglobin increased to 110 g/L by 6 months after discharge from the hospital.



Figure 1. A. lumbricoides imaged by capsule endoscopy



Figure 2. Acute bleeding seen by capsule endoscopy



Figure 3. Double balloon endoscopy demonstrating A. lumbricoides in proximal jejunum



Figure 4. The body of the A. lumbricoides retrieved from the jejunum by forceps

Discussion

Intestinal ascariasis has been recorded as early as the time of the ancient Greeks. It is still commonly seen in developing countries associated with poor hygiene and low socioeconomic status [3]. It is transmitted through an oral-fecal route by ingestion of water and food contaminated by eggs. Upon ingestion, the eggs hatch into larvae, which grow in the respiratory system, and are swallowed into the gastrointestinal tract to become mature worms in jejunum. These days, ascariasis infestation usually does not cause severe complications because of the common use and the success of antihelminthic agents. However, some severe complications such as biliary ascariasis, small bowel obstruction, massive gastrointestinal bleeding, perforation, volvulus, and necrosis of the bowel still can be found in the literature, especially in cases of high worm load and in children [4]. In terms of gastrointestinal bleeding, A. lumbricoides is a well known cause of iron deficiency anemia [6]. The organism is known to consume nutrients ingested by the host [6], but does not primarily consume red blood cells like other helminthes, such as Necator americanus and Ancylostoma duodenale [6]. However, massive gastrointestinal bleeding has only been rarely reported. Significant bleeding in infants caused by duodenal and gastric ulcers has been reported. Intestinal ulcers have been reported in adults with A. lumbricoides infestation [7,8,9]. In the current case, an adult with A. lumbricoides in the jejunum presented with acute gastrointestinal bleeding. Because A. lumbricoides possess two suckers, oral and ventral [6], which facilitate attachment to host intestinal mucosa and to prevent expulsion, it is possible that the acute bleeding in the small intestine in the current case could have been caused by mucosal damage (ulcers or erosions) due to the suckers. Such ulcerations have been described previously by Lukashok et al. However, no ulcer, erosion, or any certain bleeding point was found by capsule endoscopy or double balloon endoscopy. After removal of the worm, the bleeding promptly ceased which supports the conclusion that the bleeding was associated with the presence of A. lumbricoides. Although this patient apparently had cirrhosis, the normal coagulation tests, platelet count, lack of varices on endoscopy, and lack of clinical features of chronic liver disease suggest that cirrhosis and decompensated liver function were not present and were not involved in the presentation of acute GI hemorrhage.

Obscure gastrointestinal bleeding is a challenging condition which usually involves the small bowel. The leading causes of OGIB are small intestine tumors, angiodysplasias, small intestinal ulcers [2], and these can now be detected by capsule endoscopy, double balloon endoscopy, and helical endoscopy [2]. Capsule endoscopy is a pill-shaped device that obtains digital images of the GI tract and transmits them wirelessly for evaluation [5]. However, capsule endoscopy is currently only useful for diagnostic purposes as it does not have therapeutic capabilities [4]. However, double balloon endoscopy can be advanced deep into the small intestine and has therapeutic capabilities. As demonstrated by this current case, the sampling capability of the instrument was useful in making the diagnosis by recovery of the adult parasite.

Authors' Contribution

Fan Zhang drafted the manuscript. Li-bo Wang provided the endoscopy examinations. Yan Tan provided and followed the case. All authors read and approved the final manuscript.

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Wormy ear: A case report

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Abstract

Introduction: Myiasis is an infestation of live humans and vertebrate animals with dipterous larvae which, at least for a certain period, feed on the host's dead or living tissues, liquid substances, or ingested food. Adult female flies leave their larvae on abrasions and cuts, eyes, ears, noses and, the hollow organs, such as anus, of humans and animals. The larvae develop in organs in a short time. We report in this paper a larval infestation of Wohlfahrtia magnifica behind the right ear of a 51-year-old male.

Case report: A 51-year-old male patient presented to our emergency department with an open wound behind the right ear for one month. Physical examination revealed live maggots on the ear. The maggots were approximately 8-10 millimeters long. The patient's lesion was consulted to our clinic with right ear turned into open sores about the size of 10x6 cm. On examination, an open wound in the right ear was discovered which was covered with granulation tissue with many freely moving live larvae. In morphological examination larvae belonging to species Wohlfahrtia Magnifica were identified. Squamous cell carcinoma was diagnosed by histopathological examination of specimens.

Conclusion: Systemic examination of the patients with chronic wounds, especially those who are neglected and have poor hygiene, should be carefully done and myiasis should always be considered in differential diagnosis.

Key words: Myiasis, Wohlfahrtia Magnifica.

Introduction

Myiasis is an infestation of live humans and vertebrate animals with dipterous larvae which, at least for a certain period, feed on the host's dead or living tissues, liquid substances, or ingested food.¹ It is a worldwide infestation with seasonal variation. There is a higher incidence in the tropical and subtropical regions of Africa and America. The disease-producing flies prefer a warm and humid environment; thus, myiasis is restricted to the summer months in temperate zones, while occurring year-round in the tropical areas.² Wohlfahrtia Magnifica of the family Sarcophagidae is the predominant causative agent of myiasis in humans.¹

Adult female flies leave their larvae on abrasions and cuts, eyes, ears, noses and, the hollow organs, such as anus, of humans and animals. The larvae develop in organs in a short time.² Myiasis produces symptoms in various organ systems. Aural myiasis has a wide clinical spectrum, from maggots in the ear to otalgia, otorrhea, perforation of the eardrum, bleeding, itching, tinnitus, furuncle of the external ear, and restlessness.³ In the present paper we report a larval infestation of Wohlfahrtia Magnifica behind the right ear of a 51-year-old male.

Case report

A 51-year-old male patient presented to our emergency department with an open wound behind the right ear for one month. Physical examination revealed live maggots on the ear (Figure 1). The maggots were approximately 8-10 millimeters long. No other pathological findings were observed upon physical examination of the patient.

Patient presented to our department with a small ulcer in his right ear having begun about one month before. The lesion had grown slowly over days. Later, it progressed to an open wound with a size of 10X6 cm (Figure 2). Physical examination revealed an open wound covered with granulation tissue with many freely moving live larvae. In morphological examination, larvae belonging to species Wohlfahrtia Magnifica were identified. External auditory canal and middle ear were normal under microscope. The appearance of the wound and the specimens taken from the wound tissue were suspicious for malignancy. The patient's ear was amputed with a 2 cm surgical safety margin. Squamous cell carcinoma was diagnosed in histopathological examination of specimens.



Figure 1. Examination of the live maggots on the wound



Figure 2. General view of the wound

Discussion

Magnifica has been generally reported in humans. The clinical manifestations of the disease include ophthalmomyiasis, orotracheal myiasis, external genital myiasis, vulvar myiasis, otomyiasis, mastoidectomy cavity, and cutaneous myiasis.¹

Wohlfahrtia Magnifica larvae infest the ear, eye and nose, damaging living tissues. It lives in southeastern Europe, southern and Asiatic Russia and the Mediterranean basin. The infestation is most often subcutaneous and produces a furunculous or abcess-like lesion; however, it is also known to occur in wounds and certain body cavities.² Myiasis is closely linked with personal hygiene.³ Aural manifestations have been reported in neglected chronic lesions of patients with poor personal hygiene, children, and mentally retarded adults.^{4,5} Our patient was a 51-year-old mentally retarded male having an open wound behind the ear for a long time.

The most common signs and symptoms of aural myiasis are maggots in the EAC, aural malodorous otorrhea (purulent secretion in the EAC), perforation of the tympanic membrane, bleeding, hearing impairment, otalgia, and pruritus.^{5,6} In our patient, a foul-smelling, purulent secretion was present in the right ear, but the tympanic membrane was intact.

Although uncomplicated myiasis is a self-limiting disease (maggots leave their host when they are fully mature), it can be associated with serious and sometimes fatal complications.⁵ Therapy of aural myiasis consists of removing the maggots, local disinfection, and antibiotic treatment for secondary infections.⁵ Orbital, auditory, and nasal myiasis can cause extensive necrosis and tissue destruction and require immediate removal of the infestation.^{1,6,7} Complications such as lesions of the tympanic membrane, involvement of the middle ear, or destruction of the petrous bone require surgical intervention⁵. In our case, right ear was amputated due to the malignancy and necrosis.

Conclusion

Systemic examination of the patients with chronic wounds, especially those who are neglected and have poor hygiene, should be carefully done and myiasis should always be considered in differential diagnosis.

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A cervical hematoma secondary to spontaneous papillary thyroid carcinoma rupture

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Abstract

Background and aim: Spontaneous cervical or mediastinal hematomas have been found to arise due to tumor bleeding, a dissected aneurysm of neck vessels, bleeding and clotting disorders, spontaneous rupture of the superior or inferior thyroid artery, or nodular goiters. Here, a case of papillary thyroid carcinoma that underwent spontaneous rupture and presented as a large neck mass is reported.

Materials and methods: Emergency sonographic, chest radiograph, computed tomography (CT), fiber laryngoscopy, and blood cell count monitoring were conducted when the patient presented at a hospital with an enlarged neck mass and dysphagia. Subsequently, an urgent tracheotomy and endotracheal intubation were needed to secure the airway. Emergency neck exploration was performed.

Results: A large, organized hematoma was found in the parapharyngeal space and was resected. In addition, a firm mass was found in the left lobe of the thyroid, and the upper-left pole of this mass was ruptured.

Conclusions: Bleeding from a ruptured papillary thyroid carcinoma should be considered as a diagnosis of acute neck swelling. Imaging studies, including ultrasound, CT, and fiber laryngoscopy, as well as blood cell count monitoring, will allow a differential diagnosis to be made, and will provide additional information for the selection of a possible treatment.

Key words: Papillary Thyroid Carcinoma, Rupture, Spontaneous, Cervical Hemorrhage.

Case study

A 41-year-old woman initially presented to our outpatient department with a sudden onset of neck swelling over the previous three days, and dysphagia over the past two days. The neck mass had been present for the past two months, and the patient had a history of hypertension over the past

10 years. The hypertension had been irregularly treated, and the highest blood pressure reading previously recorded was 180/100 mm Hg. The patient denied any symptoms of an upper respiratory tract infection, a history of trauma, past operations, ingestion of any foreign objects, bleeding diathesis, or other medical problems. A physical examination found her body temperature to be 36.6 °C, with a regular pulse rate of 87 beats/min, a respiratory rate of 18 breaths/min, and a blood pressure reading of 220/140 mm Hg. In addition, the patient was afebrile, eupneic at rest, and without stridor. Clear bruising was observed over the front of the neck and the upper chest (Figure 1). Large, firm, painless, and non-fluctuant swelling was also observed on the left side of the neck. The swelling extended from the mandible to the clavicle, and the trachea was shifted to the right. An emergency sonographic examination was performed using an HDI 5000 scanner and an L12-5 linear-array transducer (GE LOGIQ S6, GE LOGIQ P5, American GE Co.). A large, soft tissue mass exhibiting a nonhomogeneous echotexture was detected in the cervical. These findings were consistent with a large hematoma present in the muscular planes. An ultrasound scan detected a total of four masses, with the largest one exhibiting inhomogeneous hypoechoic lesions with irregular shapes, unclear boundaries, posterior echo attenuation in the right thyroid lobe provided a transversal diameter of 1.4 cm. The diagnosis for this mass was a thyroid gland carcinoma or neck malignancy. Moreover, due to the swelling present in the thyroid lobe, the ultrasound scan failed to detect an additional lesion that was present and was subsequently confirmed during surgery. The undetected lesion was present in the left thyroid lobe, and had a transversal diameter of 2.5 cm. A chest radiograph also revealed an increase in soft tissue density over the left lower neck with displacement of the trachea toward the right side. To establish the extension of the process, a cervicothoracic comput-

ed tomography (CT) scan with intravenous contrast was performed. The CT scans obtained showed a hematoma-like lesion present, with most of the lesion in proximity of the left side of the neck and measuring $8.5 \times 8.0^3 \times 7.5$ cm. Furthermore, this lesion was responsible for the distinct dislocation of the trachea to the right side (Figure 2). The lesion extended from the mandible to the supraclavicular fossa within the tracheal anterior diastema, cervial anterior diastema, and paracervial diastema, and was associated with a CT attenuation number of approximately 40 Hounsfield Units (HU). In the right thyroid lobe, several masses were detected with hypodense and hyperdense calcifications. Moreover, one oval mass, measuring $2.6 \times 2.8 \times 2.5$ cm³, exhibited mixed density and was observed to be infiltrating the thyroid capsule of the left thyroid lobe (Figure 2). Accordingly, significant airway compression was observed, and compromise of the thyroid capsule in the upper-left pole of the mass was observed which extended to the large hematoma present in the muscular planes (Figure 2). A fiber laryngoscopy showed diffuse congestion and swelling of the epiglottis and bilateral pharyngeal wall had occurred, accompanied by obliteration of the glottis and pyriform sinuses. Laboratory data revealed a gradual decrease in hemoglobin levels, although other blood cell counts, blood biochemistry, thyroid function tests, prothrombin time, and activated partial thromboplastin time were all within normal ranges. While the patient was monitored in the hospital, the patient experienced dyspnea due to increased swelling and airway compression. Correspondingly, orotracheal intubation was difficult, with two failed attempts by an experienced anaesthetist. Therefore, an urgent tracheotomy and endotracheal intubation were performed to secure the airway. Emergency neck exploration was then conducted, and removal of the hematoma, as well as a total thyroidectomy, were performed under general anesthesia.

A large, organized hematoma in the parapharyngeal space measuring approximately $8.0 \times 8.0 \times 7.0$ cm³ was resected. Additional hematoma masses were found to extend from the mandible to the supraclavicular fossa within the tracheal anterior diastema, the cervical anterior diastema, and the paracervical diastema.



Figure 1. Images of the bruising observed over the front of the neck and upper chest of the patient



Figure 2. Several masses were detected in the left and right thyroid lobes. The two masses are indicated with white and black arrows, respectively

Several masses were also found in the right lobe of the thyroid. In addition, a firm mass measuring $2.5 \times 2.0 \times 2.0$ cm³ was found in the left lobe of the thyroid. This mass was ruptured in the upper-left pole and had invaded the pretracheal muscles. A pathologic examination determined that a bilateral papillary thyroid carcinoma had infiltrated the thyroid capsule, with measurements of $2.5 \times 2.0 \times 2.0$ cm³ (left) and $1.2 \times 1.0 \times 1.0$ cm³ (right). A nodular goiter was also found in the right lobe of the thyroid. Post-surgery, the hospital stay for the patient was uneventful, and the patient was discharged 14 days later. At the time of discharge, no bleeding, dyspnea, or dysphagia were observed. One month later, the tracheostomy had closed. The patient received an iodine isotope (¹³¹I) at a dose of 80 mCi, and subsequently has taken Euthyrox for endocrine suppression (150 μ g/day). After 26 months of follow-up, the patient remained recurrence-free.

Discussion

Progressive, obstructive spontaneous cervical or mediastinal haemorrhage is a serious and potentially lethal event. It has previously been reported that hemorrhage can be triggered by trauma^[1], tumor bleeding^[2], a pseudoaneurysm of neck vessels^[3], bleeding and clotting disorders, spontaneous rupture of the superior thyroid^[4] or inferior thyroid^[5] artery, a nodular goiter^[3], or high blood pressure in patients with diagnosed or undiagnosed thyroid disease, which may also be associated with other factors^[6]. To the best of our knowledge, this is the first report of a spontaneous rupture of a papillary thyroid carcinoma.

Differential diagnoses of cervical masses can involve benign and malignant neoplasms, inflammatory lesions, or a hematoma. For cervical masses due to spontaneous hemorrhage, this represents a rare and life-threatening condition due to the potential for cerebral hypoxia and airway compromise^[7]. Moreover, the resulting cervical hematoma is usually characterized by tracheal, esophageal, and vascular compression, tracheal displacement, and subsequent endolaryngeal and subcutaneous bruises^[8]. In general, the initial limited extravasation is followed by sudden enlargement and can lead to suffocation and death if untreated^[4]. In the present case, the patient presented with a large neck mass associated with a sudden onset of neck swelling and dysphagia. The cervical hemorrhages experienced by this patient were due to the spontaneous rupture of a papillary thyroid carcinoma. The reasons for this included: 1) a large tumor had infiltrated the muscles around the thyroid, thereby destructing the thyroid capsule; 2) the tumor was located in the upper part of the thyroid where the local blood supply to the thyroid is abundant; and 3) the patient's hypertension (recorded at 220/140 mm Hg in the hospital) promoted rupture of this tumor.

Based on our experience, if a patient presents with a neck mass and remains stable without progression of symptoms, imaging and clinical analyses should be conducted before determining a treatment approach. Moreover, observation may also provide valuable information as well^[5]. If respiratory failure occurs, the airway should be checked and the patient stabilized^[9]. In the present case, optic fiber laryngoscopy was used to indicate the condition of the airway and to determine whether orotracheal or nasotracheal intubation was necessary. Intubation was found to be necessary, and fiber laryngoscopy facilitated the procedure. It is recommended that fiber laryngoscopy be performed prior to conducting any further exploration under general anaesthesia. If the airway is patent, guided intubation will be possible. If intubation is not successful, then a tracheotomy may be performed. However, this situation is particularly severe given the difficulties in accessing the trachea^[9], although a tracheotomy is generally a safe approach when the cause of bleeding is unknown. For a stable patient with a stable hematoma, observation is the best option, even for cases involving a mediastinal hematoma^[5]. However, it is important to note that in two previous cases of spontaneous neck hematoma^[4], observation without exploration resulted in patient death. In the present case, since the patient's swelling was progressively increasing, leading to the deteriorating condition of the patient, emergency exploration was the best option. Moreover, based on the recommendation by Stenner et al.^[4] to use a liberal incision for the exploration of a hematoma after mobilizing the adjacent structures, this approach was also used in the present case and was found to be helpful.

Conclusion

Although bleeding from a ruptured papillary thyroid carcinoma is rare, it should be considered as a potential diagnosis for acute neck swelling. Imaging studies (including ultrasound, CT, and fiber laryngoscopy) and blood cell count monitoring can provide a differential diagnosis, as well as provide additional information for determining possible treatments.

Acknowledgments and Disclosures

This study was approved by the ethics committee of our hospital and informed consent was obtained from all participants. No conflict of interest exists for any of the authors. We thank Medjaden Bioscience Limited for assisting in the preparation of this manuscript.

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An undesirable effect of measles-mumps-rubella vaccine Herpes zoster: Case report

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Abstract

Herpes zoster (shingles) manifests as vesicular rash that is one or more dermatomal in distribution and usually occurs in adults, also impairs the life quality of the patient. It is very rare in otherwise healthy children. The cell-mediated immune responses appear to play a role in the development of shingles. It has a higher incidence in immuncompromised people. The measles and rubella vaccines which are recommended in combination with mumps vaccine for routine immunization may suppress non-specific cell-mediated immunity. Here, we wanted to report a 12-month old healthy boy who presented with herpes zoster following measles-mumps-rubella vaccination and to emphasize that this disease can occur after vaccination.

Key Words: Child, immunosuppression, measles, rubella, herpes zoster.

Introduction

Varicella zoster virus (VZV) is a double-stranded DNA virus of the Herpesviridae group. The primary infection is manifested as chickenpox and results in establishment of a lifelong latent infection in dorsal root or cranial nerve ganglia. Reactivation of the virus results in herpes zoster (shingles). Herpes zoster is characterized by unilateral papulovesicular rash localized on the skin throughout one or more sensory nerves (dermatome) (1). Herpes zoster is a disease that occurs usually in adults and very rare in childhood period. It can be determined more frequent in children in case of malignancy, immunosuppression or during immunosuppressive drug use (2). The impairment of cell-mediated immunity is responsible for the development of disease. Hence it is uncommon in healthy children (3).

Measles, mumps and rubella (MMR) are diseases that may result in severe sequels and even mortality. With the establishment of regular vaccination programs, the incidences of these vaccinepreventable diseases have significantly declined in developed countries. MMR vaccine is safe in terms of unanticipated side effects (4). However, it is known that measles and rubella vaccines temporarily suppress non-specific cell–mediated immunity (5-6). In this article, with the presentation of a case who developed herpes zoster infection after MMR vaccination, we aimed to emphasize the clarification of vaccination status of children while investigating etiologic causes of herpes zoster in healthy children. This is the first pediatric case who developed herpes zoster after MMR vaccine in the English literature.

Case Report

A 12-month old boy was admitted on November 2011 with fever and unilateral rash, erythema, and pain on the dorsal and abdominal skin, which suddenly appeared 2 days ago. Besides no evidence for immune system deficiency, he had no history of anti-neoplastic or immunosuppressive drug therapy. A febrile illness or eruptions and an upper respiratory tract infection-like symptom were not revealed in his anamnesis within last 2 months. According to the history a chickenpox infection did not occur in the patient and his family. But, it was learned in child of the neighbor, existence of history of chicken pox, recently. The patient had been given MMR vaccine 10 days ago.

On physical examination, his development was consistent with his age. There were pleomorphic vesicular lesions (some of which had become infected) spreading over the dermatomal area of thoracic sensory nerve without invading beyond the midline on the dorsum and abdomen and the patient had a subfebrile fever (Figure 1, 2). The rest of physical examination was normal. On laboratory analyses, hemoglobin was 12,1 g/dl, leukocyte count was 11000/mm³, thrombocyte count was 165000/mm³ and erythrocyte sedimentation rate was 18 mm/ hour. On peripheral blood smear evaluation, there were 42% polymorphonuclear leukocyte, 12% band cell, 38% lymphocyte, and 8% lympho-monocytic cells. There were no atypical cells. The levels of serum transaminases and serum Ig G, A, M levels were normal for his age. Anti HIV antibody test was negative, VZV IgG was positive.



Figure 1 and 2. Pleomorphic vesicular lesions with spotty infection over the right inferior thoracic dermatome area

The patient was given oral acyclovir treatment during 7 days. On the 3rd day of the treatment, the lesions began to wane and the symptoms of discomfort and fever resolved. Any new lesion formation or complications were observed during visits.

Discussion

Varicella zoster virus (VZV) is an enveloped double-stranded DNA virus of human Herpesviridae family; the virus becomes latent with location in in dorsal root ganglia and subsequent reactivation causes herpes zoster. The impairment of non-specific cell-mediated immunity is much more likely to be the cause of the disease. In immuncompromised people, the incidence of the disease is significantly higher and the clinical presentation may also become more severe. Previous chickenpox infection, vaccination, immunosuppressive drugs, chronic steroid use, AIDS, bone marrow/organ transplantation, cancer, and trauma are the risk factors for zoster reactivation (7). In children who are immuncompromised or under immunsuppressive therapy and those having chickenpox in infancy, those having the virus transplacentally, and the children who are intensely exposed to the virus are at particular risk. Rarely, the disease occurs in healthy children and usually it has a mild course (3-6). Chronic stress and child abuse may be the reason of herpes zoster in healthy children (8). Chronic psychological stress adversely affects the cell-mediated immune response, so that it can be a predisposing factor for the development of herpes zoster (9). The patient was living in a family of moderate socioeconomic status without emotional stress. His development was consistent with his age. There was no history of immunosuppressive drug use or chickenpox infection before. Except in childhood period there was no chickenpox infection history in his family and no direct contact exposure history. The MMR vaccination 10 days ago was the only risk factor for the patient.

The diagnosis of herpes zoster is made by classical prodromal symptoms of pain-burning and zona rash. The rash appears unilaterally following the dermatomal distribution (10). In the presence of atypical rash, cytopathologic evaluation and polymerase chain reaction may be helpful (11-13). The virus may be present within the lesions for a few days, cutaneous dissemination is not common in individuals without immunosuppression (11). A cutaneous dissemination was not observed in the patient.

Differential diagnosis should include herpes simplex and bullous dermatoses. Current laboratory diagnostic methods for VZV are polymerase chain reaction, cell culture, determination of VZV-specific antibodies (12). In our patient, the diagnosis was made by clinical signs and positive VZV IgG test.

Herpes zoster affects quality of life, because the local pain is severe. It is thought that pain results from the inflammation of receptors and stimulation of the primary neurons with tissue injury (8). Systemic antiviral therapy alleviates the disease symptoms and is recommended particularly for older patients and immuncompromised patients. The goal of the treatment is to provide rapid healing, pain control, and to reduce as much as possible the risk of complication. Especially acyclovir is widely and safely used in pediatric patients (12). Early administration of antiviral therapy shortens the healing time of rash, reduces the severity of the disease and complications (13). We administered acyclovir treatment to our patient during seven days without any complication and we achieved a good response.

In our country, the measles vaccine in the routine vaccination schedule was substituted with MMR vaccine in 2006. MMR vaccination is given in two doses, first at 12 months of life and the other in the first class of elementary school (6-7 years of age). Since all three components of MMR vaccine consist of live viruses, clinical presentations of natural infections of these agents such as parotitis, arthralgia, and meningitis are the most important adverse effects of the vaccine. However, these manifestations after vaccination occur less often and have a mild course in comparison with natural infections. Among neurologic side effects, the most common one is aseptic meningitis occurring due to the mumps component (13). Arthralgia and arthritis, which are the most important adverse reactions of rubella vaccine, generally occur in young women (14,15). Besides, it has been reported that several adverse reactions such as hypersensitivity reactions, thrombocytopenia, encephalitis or encephalopathy may be seen after MMR vaccination, however, this vaccine is not associated with Crohn's disease, ulcerative colitis or autism (15).

The most common systemic side effect is fever occuring 5-12 days after vaccination and lasting one or two days, which is due to measles component. High fever may cause febrile seizures in young children (15). Particularly measles and rubella vaccines may cause immunosuppression through inhibiting cell-mediated immunity, which is characterized by defective lymphocyte response against mitogens, transient lymphopenia and defective cytokine production. This effect begins about one week after vaccination and lasts at least one month (5-6). Thus, tuberculin reaction is erroneously found to be negative. Given the patient presented here, we think that clinical onset of herpes zoster 10 days after the vaccination is due to the immunosuppressive effect of the vaccine.

The case presented here is the first herpes zoster case developed after MMR vaccine; the patients presenting with herpes zoster might be asked if there is a recent MMR vaccination.

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Successfully management of survival-threatening hemodialysis vascular access dysfunction: A case report

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Abstract

The present report describes a case of survivalthreatening hemodialysis vascular access dysfunction (HVAD) in a 67-year-old woman with established chronic kidney disease stage 5 for twelve years. On admission, the patient endured a heavy history of multiple major vessels damage, presenting as bilateral internal jugular vein occlusions, bilateral innominate vein stenoses, the right external jugular and femoral venous thromboses. Furthermore, the left femoral vein catheter was undergoing diminished blood flow rates and requirement for urokinase which resulted in an emergency request for a new functioning vascular access. Hence, with a digital subtraction angiography (DSA)-guided percutaneous transfemoral approach, the right external jugular tunneled catheter placement was performed following balloon angioplasty. At two-week follow-up, the substantial improvement demonstrated that the right external jugular tunneled catheterization would be a feasible and potentially optimal intervention for HVAD when routine approaches were excluded.

Key words: Hemodialysis vascular access dysfunction, end-stage renal disease, peritoneal dialysis, chronic kidney disease, percutaneous transluminal angioplasty.

Introduction

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Hemodialysis vascular access dysfunction (HAVD) is one of the leading causes of morbidity and hospitalization among the end-stage renal disease (ESRD) patients. According to Medicare data, it has been estimated that HAVD is responsible for 20% of all hospitalizations in the hemodialysis population at cost of approximately USD 1 billion per

annum[1, 2]. Despite of systematic observation and advanced surveillance have been employed to predict and prevent access failure, HAVD remains a thorny problem. Notably, vascular accesses get damaged and exhausted gradually during long-term hemodialysis. Complicated with diabetes mellitus, obesity, or atherosclerosis, the long-term hemodialysis patients even have to fight for survival when HAVD occurred or flared up. In order to prolong functioning vascular access and improve quality of life, it demands increasingly safe and effective management strategies for HAVD.

Herein, we report a case with survival-threatening HVAD which was successfully solved by placement of the right external jugular vein (REJV) tunneled catheter after balloon angioplasty under digital subtraction angiography (DSA) guidance.

Case report

A 67-year-old female patient was admitted to the department of Nephrology with established chronic kidney disease stage 5 (CKD5) and maintenance hemodialysis for twelve years, followed by HVAD flare for three days. During the previous three-year period, the patient had undergone two procedures of native arteriovenous fistula (AVF) on the left arm and one tunneled catheter via the right internal jugular vein (RIJV) due to failures or thrombosis. Then, she changed towards peritoneal dialysis (PD) which had kept working for six years. Owing to inadequate dialysis and peritonitis, she opted for the reentry of hemodialysis. Unfortunately, the following polytetrafluoroethylene (PTFE) arteriovenous grafts (AVG) ended in primary failure because of thrombosis. Afterwards, hemodialysis was successively performed by tunneled central vein catheter via the

left internal jugular vein (LIJV) and the right femoral approach. On the latest admission, the tunneled femoral vein catheter developed thrombosis again. Moreover, she suffered from type 2 diabetes mellitus, chronic heart failure, left ventricular enlargement, renal hypertension and renal anemia from the previous years. On physical examination, her body mass index (BMI) was 38. She had a puffy face with dusky discoloration and pitting edema in her lower legs. Color Doppler ultrasound showed a right femoral thrombus within the catheter, thickening vessel wall of the left femoral vein, and bad occlusion of RIJV, LIJV and REJV. Vascular three-dimensional reconstruction of chest computed tomography revealed the left brachiocephalic vein and bilateral internal jugular vein stenosis.

Before AVG could be performed, her chronic heart failure aggravated suddenly. Under this situation, options such as AVF, AVG, PD and transplantation were excluded. However, reestablishment of a functioning long-term vascular access appears difficult but pressing. That was threatening the survival of this patient. Fortunately, DSA-guided percutaneous transfemoral approach was utilized. Angiograms obtained from DSA manifested stenosis or occlusion of the left brachiocephalic vein, REJV, RIJV near to venous angle, and even the right brachiocephalic vein (Figure 1 A). After balloon angioplasty to RIJV and the right brachiocephalic vein, RIJV tunneled catheterization was tried. Unfortunately, it failed because the recovered vein got restenosis before catheter insertion. Since the placement of RIJV tunneled catheter was unavailable, we opted for REJV as catheter insertion site. Under DSA guidance again, tunneled catheter was implanted via REJV approach followed with successful balloon angioplasty to REJV and right brachiocephalic vein (Figures 1 B, 1 C). Meanwhile, pharmacological administration was given to prevent thrombosis, delay progression of renal damage and correct the hypertension, anemia and metabolic disorder of renal failure. At twomonth follow-up, a sufficient extracorporeal blood flow (\geq 300mL/minute) confirmed the successful management of such survival-threatening HVAD.

Discussion

HAVD management is pivotal in maintaining the survival and the quality of life in long-term hemodialysis patients. To optimize access longevity and minimize HAVD, the Kidney Disease Outcomes Quality Initiative (K-DOQI) summarized clinical practice guidelines for HAVD management[3]. For long-term hemodialysis accesses, AVF should be the preferred type and catheter should be used only when AVF or AVG are not available. In our case, the patient was supposed to choose catheter in case that heart failure is a con-



Figure 1. (A) It is showed that the bilateral brachiocephalic vein, RIJV near to venous angle and REJV were occlusive or stenotic. (B) Angiogram construction of expanded right brachiocephalic vein. (C) Insertion of the RIJV tunneled catheter after balloon angioplasty.

traindication to AVF or AVG creation, plus that catheter usually could be utilized immediately. Of important note, she suffered from multiple risk factors such as diabetes mellitus, obesity, old age and venous thrombosis history, which entitled her with high risk for AVF and AVG failure[4].

As the catheter population is growing, catheter dysfunction highlights the significance of HAVD management. Researches demonstrated that tunneled catheters have a better blood flow characteristic and a reduced risk of infection than non-tunneled catheters[5]. RIJV, as the preferred placement site, can also contribute to minimization of HAVD. However, as vascular access sites become progressively depleted, RIJV location is always unavailable because of thrombosis or stenosis, and then alternate location must be sought. Although the most common choice is LIJV, recent studies indicated REJV to be an acceptable and preferred location when RIJV is not accessible[6]. The advantage of REJV catheters over LIJV ones is better preservation for the left sided vasculature in general, especially when future left-sided access is being planned[7]. In addition, the blood flow rates of REJV catheters were comparable to LIJV and RIJV catheters[8, 9]. Our experience also suggested REJV to be an alternate location when RIJV is not accessible.

Confronted with exhausted major vessels, multiple high risk factors for access failure, and pressing access replacement, any aborted intervention for HVAD management may threaten the survival of long-term hemodialysis patients. Offering the advantage of imaging central veins with high sensitivity and specificity, in particular, being a prelude to direct intervention at the same sitting, DSA has not only diagnostic value in the evaluation of HVAD, but also therapeutic value in the guidance of intervention procedures[10]. To the present day, percutaneous transluminal angioplasty (PTA) is the preferred treatment for central vein stenosis. Consequently, DSAguided REJV tunneled catheter placement followed with balloon angioplasty yields a new functioning vascular access. Successful management indicated REJV tunneled catheterization could be a feasible and potentially optimal intervention for HVAD when routine approaches were excluded. However, further prospective investigation is required to establish the superiority of REJV access as well as the efficiency of DSA-guided balloon angioplasty.

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Bowen's disease associated with other skin cancers and successfully topically treated with 5-fluorouracil and 3% diclofenac gel

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Abstract

Background: Bowen's disease (BD) is a form of squamous cell skin carcinoma in situ. It usually appears as a single lesion and associations with other tumors are rare. 5-fluorouracil (5-FU) is well known therapeutic option for BD, but 3% diclofenac gel is newer treatment modality whose efficacy and optimal time of application are not yet known.

Case report: We present BD associated with squamous cell carcinoma and basal cell carcinoma in 92-year old woman. BD presented in a form of large, erythematous, scaly plaques on her left cheek which appeared 8 years before examination. According to medical documentation, squamous cell carcinoma on the forehead and basal cell carcinoma on the nose developed one year before examination, and 6 months later were operated. We noticed only postoperative skin defects within these locations.

The lesions of the BD was treated with 5-FU twice daily for 6 weeks, then 3% diklofenac gel was applied twice daily for 60 days and complete healing was achieved.

Conclusion: On this rare occasion, BD, squamous cell carcinoma and basal cell carcinoma developed in the same region. Topical combined therapy with 5-FU and 3% diclofenac gel is successful treatment option for BD.

Introduction

Bowen's disease (BD) is a form of squamous cell carcinoma in situ. It usually appears as a solitary, slowly enlarging, scaly, sharply demarcated erythematous plaque. It may affect both skin and/ or mucosa, but is most common in photo exposed regions. If left untreated, BD progresses to invasive squamous cell carcinoma in 3-5% of patients (1)(2). In most cases, BD appears as a single lesion and associations with other tumors are very rare.

There are different treatment recommendations for BD depending on localization and extent of the lesion. Topical 5-fluorouracil (5-FU) induces inhibition of DNA synthesis, creating a thymine deficiency that promotes cell death, particularly targeting rapidly multiplying tumor cells (3). Several studies have since been published supporting the usefulness of 5-FU in the treatment of BD (4)(5)(6).

It is known that the diclofenac 3% gel is well tolerated and efficient in actinic keratosis (7). Only a few authors indicate that it is also effective in BD (8)(9).

We present a rare case of BD on the face in 92-year old woman, associated with sqamous cell carcinoma and basal cell carcinoma and successfully topically treated with 5-fluorouracil and 3% diclofenac gel.

Case report

A 92-year old women was examined for the evaluation of the lesion on the her left cheek which appeared 8 years ago and slowly enlarged. History and medical documentation suggested that she underwent surgical excision of the forehead and nose tumors 4 weeks ago. The tumor of the forehead appeared a year ago and has grown into a large, prominent mass. Histology revealed squamous cell carcinoma. The lesion of the nose, presented as a nodule, appeared 8 months ago. Histopathology has shown basal cell carcinoma. On examination, we noticed a postoperative skin defect of 8x6cm in diameter on the forehead and 2x2cm on the nose (Figure 1). On the left cheek, two erythematous plaques sized 6x5 cm and 2x2 cm were seen. Plaques were

sharply demarcated and partially covered with white scales (Figure2). Histopathology examination suggested BD with light hyperkeratosis, epidermal atrophy and atypical squamous cell proliferation. The dermo-epidermal junction was retained. In the dermis, thick, banded lymphocyte infiltrate was observed. (Figure 3). Computed tomography did not detect changes in the neck lymph nodes. Parotid and submandibular nodes were of normal size and homogenous. Biochemical analyses of the blood and urine were within normal limits Chest and abdomen computed tomography did not show metastatic lesions or lymphadenopathy.



Figure 1. Scars after surgery of squamous cell carcinoma on the forehead and basal cell carcinoma on the nose



Figure 2. Well- demarcated erythematous, scaly patches of Bowen's disease on the left cheek

Treatment was initiated with topical 5-fluorouracil (5% concentration of 5-FU in propylene glycol) applied twice daily for 6 weeks, which led to partial regression of the lesion (Figure 4).



Figure 3. Histopathology revealed hyperkeratosis, epidermal atrophy, pathological mitosis, atypia and stratification disturbance of keratinocytes. Tumor cells were not found in the dermis.

Two residual patches of erythema and scaling measured approximately 2x2 cm persisted (Figure 3). No significant side effects were noticed. Then, remaining lesions were treated with 3% diclofenac gel twice daily for 60 days and complete healing was achieved. Post treatment skin biopsy showed no evidence of recurrence of BD. The patient has been followed up for 12 months and remains clinically free of disease.



Figure 4. Lesions of Bowen's disease after treatment with 5-fluorouracil



Figure 5. Complete healing of Bowen's disease after treatment with 3% diclofenac gel

Discussion

Bowen's disease was first described in 1912. by JT Bowen. Clinically, a typical BD is a gradually enlarging, solitary erythematous plaque with clear borders and scaling or crusted surface. It occurs more often in elderly patients; irradiation, carcinogens like arsenic, immunosuppression, certain types of human papiloma virus, chronic injury, and rarely, preexisting seborrhoeic keratosis, have been implicated as etiological factors for developing BD (2).

Its distribution is consistent with chronic sun exposure, being more common on the head and neck in men, and lower limbs and cheeks in women. Rarely BD may occur in perianal region (6), subungually (10), or on eczematous skin (11). Histopathologic examination remains the gold standard for diagnosis of BD (12). Bowen's disease has been repoted to appear in combination with other malignant tumors in only few cases

(13). Matsumoto C. et al. (14) presented two patients afflicted with both squamous cell carcinoma (SCC) and BD on the same parts of the body, namely SCC on the left foot and BD on the right foot. Bowen's disease and basal cell carcinoma in a patient with SCC of the lung has been reported by Kim HJ et al (15). We presented a rare case of BD associated with squamous cell carcinoma and basal cell carcinoma in the same region. According to the guidelines (2), BD can be treated by surgery, curettage and electrocautery, cryotherapy, 5-fluorouracil, laser therapy, radiotherapy, photodynamic therapy, imiqimod or local or systemic injections of interferon alpha or gamma. Despite of the wide range of modalities currently available, the removal of the large, poorly healing lesions (regarding increasing age of the patient, increasing lesion diameter, and higher radiation doses) of BD is very difficult.

We reported treatment success of the large BD on the face in 92-year old woman with topical combined therapy of 5-fluorouracil (5-FU) and 3% diklofenac gel.

Although excision using the Mohs technique achieves a high cure rate, surgical removal may result in significant disfigurement, especially if the lesions are extensive, or in cosmetically sensitive areas.(16)(17). Also, we believed that a trial of combination therapy would be useful for the patient if monotherapy failed to elicit a quick response. Topical 5-FU was first used in the early 1960s as experimental treatment for various skin lesions. Dillaha et al. (18) described the selective effect of topical 5-FU on actinic keratoses with minimal effect on surrounding normal skin. This selective effect is primarily attributed to its direct cytotoxity to cancer cells. 5-FU is an S-phase-specific antimetabolite and structural analog of thymidine that exerts cytotoxicity primarily by irreversibly inhibiting thymidylate syntetase, which results in lower levels of thymidine monophosphate and impairs DNA synthesis in actively dividing cells, resulting ultimately in cell death (3). 5-FU may have further cytotoxic effects by being incorporated directly into DNA and RNA macromolecules, thereby inhibiting normal processing and function, including gene transcription, translation and DNA replication (16). Dillaha et al (17) also revealed success of 5-FU in the topical treatment of BD during 4 weeks. In a trial of topical 5-FU applied twice daily for 9 weeks, Bargman and Hochman (4) reported clinical success in all cases of BD. Although 5-FU is an established treatment option for BD, there is no consensus on the optimal treatment duration. In our patient, 5-FU was applied for 6 weeks, when partial healing was achieved.

In order to increase efficacy, 5-FU has been combined with multiple therapeutic modalities in

the treatment of BD including cryosurgery, imiquimod, tazarotene and dinitrochlorobenzene (19). In the same order, we combined topically 5-FU with 3% diklofenac gel. The mechanism of action of diklofenac gel is yet unknown. Epidemiological studies show that chronic inflammation predisposes patient to cancer development. Prostaglandin E2 has been shown to promote cell survival, proliferation, and angiogenesis and inhibit apoptosis and antitumor response, all processes promoting cancer development (20). Diclofenac is one of the oldest NSAIDs which inhibits cyclooxygenases, rate-limiting enzymes in the production of prostaglandins from arachidonic acid. Dawe SA et al. (8) reported two cases of BD successfully treated with 3% diklofenac gel. Both of them were treated twice daily for 90 days and complete healing was achieved, while no serious side effects were noticed. The patient has been followed up for 12 months and remains clinically free of disease.

According to our knowledge, it is the first reported case of association of BD with basal cell carcinoma and squamous cell carcinoma on the face, and first reported success of combined topical treatment of BD with 5-FU and 3% diclofenac gel.

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Fulminant type 1 diabetes presentation in China: One case report

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Abstract

Fulminant type 1 diabetes is a recently discovered subtype of type 1 diabetes, characterized as an extremely rapid process of β -cell destruction and progression to hyperglycemia and ketoacidosis. Herein, we present a case of fulminant type 1 diabetes in a 43-year-old Chinese woman, along with a review of the literature. The patient presented with sudden onset of polydipsia and polyuria followed by nausea and vomiting symptoms. Findings on admission included a high blood glucose level and ketoacidosis, but normal HbA1c level. The C-peptide stimulation test showed severe impairment of insulin secretion. Autoantibodies were all negative. These results are compatible with the diagnosis of fulminant type 1 diabetes.

Key words: Fulminant type 1 diabetes, Diabetic ketoacidosis.

Introduction

According to the classification of diabetes by the American Diabetes Association (ADA) and the World Health Organization (WHO), type 1 diabetes mellitus (T1DM) is divided into two subtype: autoimmune type 1 (type 1A) diabetes and idiopathic (type 1B) diabetes. But recently fulminant type 1 diabetes mellitus (FT1DM) is found as a new clinical subtype of T1DM that had been first reprorted in 2000[1] and which the process of β -cell destruction and the progression of hyperglycemia and ketoacidosis are extremely rapid. Although some evidences have suggested that viral infection and genetics are associated with the development of FT1DM, the pathogenesis of this type of diabetes remains unknown. A nationwide survey in Japan revealed that FT1DM accounted for 15%-20% of Japanese T1DM with ketosis or ketoacidosis at onset[2]. There was only a few cases reported in China and most of the patients were pregnant women. Here we report one case which had been observed for one year.

Case presentation

A 43-year-old Han Chinese man presented to a local hospital with nausea and vomiting several days. He complained of polyurea and polydipsia for 2 weeks. He was diagnosised as diabetes ketoacidosis and after simple therapy he was transferred to our hospital for further treatment.

The patient had a history of chronic type B viral hepatitis, and had no past medical or surgical history and a significant family history of diabetes mellitus. The patient was not on any medications prior to admission. He did not smoke, drink alcohol or engage in recreational drug use. Physical data on admission showed a height of 173 cm and a weight of 52.5 kg with a body mass of 17.5 kg/m². His cardiac, pulmonary and abdominal exams were normal.

His initial laboratory data was as follow: urinalysis showed 3+ ketones and 3+ glucose, while blood ketone were positive. Blood arterial PH was 7.28 (normal range 7.35-7.45), serum level of bicarbonate was 23.5 mmol/L (normal range 35-45 mmol/L), standard base excess (SBE) was -8.2 mmol/L (normal range -3.0-3.0 mmol/L), glucose was 16mmol/ L, whereas HbA1c was 6.1% (normal range 4.5%-6.5%). Blood amylase was 1794 U/L (normal range 17-220 U/L). Complete blood count and blood electrolyte were in normal range. All of aspartate aminotransferase (AST), alanine transaminase (ALT), albumin, sodium, urea nitrogen, total cholesterol, and triglyceride were normal. As the raised level of blood amylase, the patient received abdominal CT enhanced scan and the result had no sign for pancreatitis. Other tests such as chest X-ray, electrocardiogram, urine examinations, and serum troponin I (TNI), creatine kinase (CK), MB isoenzyme of creatine kinase (CK-MB), and lactate dehydrogenase (LDH) measurements were all negative.

According the symptoms and lab data on admission, we considered the patient to have T1DM and diabetic ketoacidosis. The patient was started on an intravenous insulin infusion at a rate of 0.1 u/kg/h.

When his blood ketone into negative, intensive insulin therapy four times a day was given. After initial treatment, his condition was stabilized gradually. Blood amylase level dropped down to normal level after therapy. Further examination showed that the autoantibodies including insulin autoantibodies (IAA), islet cell antibodies (ICA) and glutamic acid decarboxylase antibodies (GAD-Ab) were all negative. C-peptide stimulation tests showed that both of the fasting and stimulated serum C-peptide were <0.05 ng/ml after oral administration of 83.5 g glucose, which meant severe impairment of insulin secretion. Serological tests of virus antibody titers showed that immunoglobulin G (IgG) antibody titers for cytomegalovirus and Epstein-Barr virus (EBV) were 4.7 and 3.1 espectively, while all of immunoglobulin M (IgM) antibody titers for cytomegalovirus, EBV, coxsackie virus, and enterovirus RNA test were negative. Autoantibody to nuclear antigen (ANA), antineutrophil cytoplasmic antibodies (ANCA) and anti mitochondrial antibody (AMA) were all negative. When discharged from hospital, the patient had been observing for nearly two years. His blood glucose was well controled and HbA1c was contained below 6.5%. C-peptide stimulation tests was repeated for two times after half a year and two years later, which showed both of the fasting and stimulated serum Cpeptide were still <0.05 ng/ml.

Discussion

The diagnostic criteria for fulminant T1DM require: (1) evidence of ketosis or ketoacidosis within about 7 days of hyperglycaemia onset, (2) plasma glucose level greater or equal to 16.0 mmol/L (> 288 mg/dL) and HbA1c less than 8.5% at initial visit, (3) urinary C-peptide excretion less than 10µg/d or fasting serum C-peptide level less than 0.3 ng/mL (<0.10 nmol/L), or less than 0.5 ng/mL (<0.17 nmol/L) after intravenous glucagon or after meal at onset [3]. As more cases of rapid onset diabetes with ketoacidosis present, the original definition of fulminant T1DM that included lack of auto-antibodies and evidence of pancreatitis is not always fulfilled. The clinical features of the case reported here were exactly conformed to the above criteria, so the diagnoses were confirmed.

The pathogenesis of this disease involves both genetic background and viral infection. With the

majority of cases reported in the Asian population such as Japan, Korea and Philippines[4-6], some researchers have hypothesized that there is a genetic susceptibility in the Asian population. Imagawa et al.[7,8] have confirmed that in Japan, individuals with HLA DRB1*0405-DQB1*0401 are more likely to develop fulminant T1DM, whereas in Caucasian with HLA DRB1*0401-DQB1*0302 increases the risk for classical T1DM. But there has no large study to demonstrat which genetype is linked with Chinese FT1DM patients.

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Cat eye syndrome accompanied by pseudohypoparathyroidism: A case report

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Abstract

Cat eye syndrome is considered to be a rare chromosome disease. Clinically, the disease is characterized by the presence of multiple malformations, primarily involving the eyes, ears and anorectal and urogenital systems. It is caused by tetrasomy on chromosome 22. Case reported here exhibited three cardinal features of cat eye syndrome; cleft palate, ectopic kidney and interatrial septal defect. In addition, she had several minor features including microphthalmia, mild downward-slanting palpebral fissures, proptosis, hypertelorism, micrognathia and skeletal anomalies (hallux valgus). Previous genetic evaluation had revealed partial tetrasomy 22. Pseudohypoparathyroidism is an inherited disorder characterized by an end-organ resistance to parathormone. Children with pseudohypoparathyroidism present with hypocalcemia and normal or elevated serum phosphorus concentrations despite elevated serum parathormone levels. Here, a 6-month-old female patient was reported, with major and minor findings of cat eye syndrome, including pseudohypoparathyroidism. To the best of our knowledge, no case of cat eye syndrome complicated with pseudohypoparathyroidism has been previously reported.

Key words: Cat eye syndrome, pseudohypoparathyroidism, tetrasomy 22.

Introduction

Also known as Schmid-Fraccaro syndrome (OMIM 115470), cat eye syndrome (CES) is a rare chromosome disease seen in an estimated 1 in every 50,000-150,000 live births. The disease is characterized by the presence of multiple malformations, primarily involving the eyes, ears and anorectal and urogenital systems [1]. It is caused by partial tetrasomy of chromosome 22 [2]. Here, we report a 6-month-old female patient with major and minor

findings of cat eye syndrome. After additional investigations it appeared that the patient also had pseudohypoparathyroidism. Pseudohypoparathyroidism (PHP) is an inherited disorder characterized by an end-organ resistance to parathormone (PTH). Children with PHP show hypocalcemia and normal or elevated serum phosphate (P) levels despite elevated serum PTH levels. To the best of our knowledge, this is first case of the CES complicated with PHP.

Case Presentation

A 6-month-old girl infant was referred to our clinic because of vomiting, developmental regression and growth retardation. She had been born via normal spontaneous vaginal at term, weighing 2600 g after uncomplicated pregnancy and delivery. At birth, a cleft palate was diagnosed. She was fed only with formula and receiving 400 U/day vitamin D supplementation. With history, no developmental delay or other genetic disorders were detected in other members of family. On physical examination, her length, weight and head circumference were under the 3rd percentile. Anterior fontanel was open and flat, and posterior fontanel was closed. Mild downward-slanting palpebral fissures, proptosis of both eyes, flattened nasal bridge and micrognathia were noted (Figure 1). Furthermore, there was hallux valgus in both feet (Figure 2). No colobomas were noted. Her psychomotor development was delayed. The case with failure in consistently fixing and following was lethargic, not smiling and rolling over yet. She had neither signs of rickets nor others suggesting Albright's Hereditary Osteodystrophy (AHO), such as short hands, round face, central obesity or dental hypoplasia.

Laboratory investigations revealed a hemoglobin level of 7.5 g/L, granulocyte count of $16.17 \times 10^{3/2}$ mm³, and platelets of $560 \times 10^{3/2}$ mm³. Her mean corpuscular volume was 71.2 fL, red blood cell count 3.02x10⁶/mm³ and hematocrit 21,5%. The serum Calcium (Ca), magnesium and P levels were 5.4 mg/dL (reference range 9-11), 2.1 mg/dL (reference range 1.5-2.6) and 7.7 mg/dL (reference range 4-7), respectively. Serum alkaline phosphatase level was 266 U/L (reference range <750). Serum albumin level was 3.3 g/dL (reference range 3.8-5.4). Corrected serum Ca level based on the serum albumin level was 5.96 mg/dL.



Figure 1. Facial appearence of the patient. Visible features are mild downward-slanting palpebral fissures, proptosis of both eyes, flattened nasal bridge and micrognathia



Figure 2. Hallux valgus sign on left foot. Lateral deviation of the great toe is seen

When the characteristics of atypical phenotype were detected, and hypocalcemia was determined in the patient's routine biochemical assays, more detailed investigations were considered to be performed. Parathyroid hormone level was 679 pg/mL (reference range 11-67). Levels of FSH, LH, TSH and FT4 were within normal limits. Serum 25-hydroxyvitamin D and serum 1,25-dihydroxyvitamin D were 27.3 U/L (reference range 10-120) and 40.00 pg/mL (reference range 30-90), respectively.

The urinary calcium/urinary creatinine ratio and tubular phosphate reabsorption were 13.43 µg/mg (normal range: 30–810) and 89.4% (normal range: 85–97%), respectively. Renal functions were within normal limits. An ejection systolic murmur was discovered, and subsequent echocardiogram revealed secundum atrial septal defect and a left to right shunt. Renal ultrasound revealed a left ectopic kidney in bone pelvis, and collecting system was severely dilated consistent with ureteropelvic junction obstruction. On cranial CT, no calcification of basal ganglia was seen. She failed on hearing screening test performed for newborns. Her genetic evaluation revealed partial tetrasomy 22.

The patient was diagnosed with CES accompanied by PHP, based on the clinical and laboratory findings. A supplementary treatment with calcitriol and calcium gluconate was started. Then, the treatment was continued with calcitriol and calcium lactate. During the last examination of the patient followed-up over a year, levels of serum Ca and serum P were found as 9.4 mg/dl and 6.1mg/dL.

Discussion

CES is a rare chromosomal disorder that may be evident at birth. Karyotype analysis reveals tetrasomy 22. With genetic definition of CES, ocular coloboma is now known to be present in less than 50% of patients. Associated symptoms and findings may vary greatly in range and severity. In many cases, characteristic features of the disorder include mild intrauterine growth retardation, mild mental deficiency, and craniofacial, cardiac, renal and anal malformations. Specifically, individuals with CES frequently have colobomas, downward-slanting palpebral fissures, hypertelorism, and/or other ocular defects, misfigured ears, preauricular tags and cleft palate [3]. Our patient had three cardinal features: cleft palate, ectopic kidney and interatrial septal defect. In addition, she had several minor features including microphthalmia, mild downward-slanting palpebral fissures, proptosis, hypertelorism, micrognathia and skeletal anomalies (hallux valgus). Her genetic evaluation had also revealed partial tetrasomy 22.

PHP is characterized by PTH resistance (elevated PTH levels in the presence of hypocalcemia and hyperphosphatemia) associated with a blunted cAMP and phospahaturic response to exogenous PTH. PHP is mainly classified as type 1 and 2. Type I also divides into two subgroups; type Ia and type Ib. In Type Ia, affected children have short, stocky built body formation and a round face (AHO). Brachydactyly with dimpling of the dorsum of the hand is usually present. There may be other skeletal abnormalities such as short and wide phalanges, bowing, exostoses, and thickening of the calvaria. In addition to resistance to PTH, resistance to other G protein-coupled receptors for thyroid-stimulating hormone (TSH), gonadotropins, and glucagon can result in various metabolic effects. In Type Ib, affected patients have normal levels of G protein activity and a normal phenotypic appearance. These patients have tissue-specific resistance to PTH, but not to other hormones. Serum levels of calcium, phosphorus, and immunoreactive PTH are the same as those in patients with type Ia PHP. Type II has been detected in only a few patients and differs from type I in that the urinary excretion of cAMP is elevated both in the basal state and after stimulation with PTH, but no increase occurs in phosphaturia. Phenotypically, patients are normal, and hypocalcemia is present. The defect appears to be distal to cAMP because it is normally activated, but the cell is unable to respond to the signal [4].

In our case, elevated levels of serum PTH was determined, while decreased levels of serum Ca and increased levels of serum P were found. Therefore, the clinical findings and initial laboratory results suggested PHP type 1b. Our patient had no phenotypic features of AHO. The reason could be that 6 months of age is a very early period for detecting clinical features of AHO. Furthermore, Albright Hereditary Osteodystrophy findings might have been obscured by clinical features of CES. The existence of PHP in our patient was considered to be transient; however, the fact that the case was supplementarily treated with calcitriol and calcium lactate for one year suggested that PHP was a permanent condition.

CES is caused by partial tetrasomy of chromosome 22, the result of a supernumerary dicentric marker chromosome with satellites at the ends, inv dup (22) (pter >q11.2::q11.2->pter). This, as its description states, involves duplication of the entire short arm of chromosome 22 (p) plus part of its long arm (q), as far as band 11. It is now known that this band contains regions of low copy repeats which predispose it to rearrangements, including the marker chromosome observed in CES [2]. Mutations on chromosome 20 are known to be responsible from PHP. However, PHP has been reported with deletion of chromosome 22q11 and in Catch 22 syndrome [5,6]. In light of the reported cases, our patient with tetrasomy 22 is considered to have a relationship between PHP and abnormalities of chromosome 22.

In conclusion; CES is a complex, rare chromosomal disorder, involving clinical signs of various systems. As a result of decreased level of serum hypocalcemia, our patient was diagnosed with PHP, while being followed up with the diagnosis of CES. Thus, as with our case, patients with atypical facial appearance and abnormalities of chromosome 22 should be evaluated meticulously in terms of PHP and other systemic findings. To our knowledge, this is the first to report a CES case accompanied by PHP in literature.

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Metastatic melanoma in the tonsil in diffuse melanosis- case report

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Abstract

Melanoma is a neoplastic lesion, which develops from atypical melanocytes. Primary mucosal melanoma of the head and neck is an uncommon lesion. Melanoma metastasize through lymphatic and vascular routes. Most of melanoma metastasis can be detected on the basis of the appearance of symptoms or physical examination. In this paper we present a rare case of metastatic tonsillar malignant melanoma.

Key words: melanoma, metastasis, tonsils, prognosis

Introduction

Melanoma is a neoplastic lesion, which develops from atypical melanocytes of the basal layer of the epidermis and makes 1-2% of all malignant tumors (1, 2). It is the most common disease that can lead to death. Annual increase in the number of people affected by 4-5% means that the number of cases doubles every 10-15 years. The mortality from the disease is also increasing (3, 4). In this paper we present a rare case of metastatic tonsillar malignant melanoma.

Case report

Male, aged 38 years, came to examination in the ENT clinic in August 2011 with pain in the throat, difficulty swallowing, and poor general condition. We found out that he had tumor surgery on the forearm of his right hand in January 2008 and that was histologically verified as melanoma. He had radiation treatment, chemotherapy, interferon for metastatic changes in the lungs, adrenal glands, liver, endocranium, after previous ultrasound and radiology diagnostics (computed tomography and magnetic resonance). He underwent surgery in May 2010 due to secondary deposits in the stomach and small intestine. Immunohistochemical phenotype of tumor cells were HMB45 Melanome positive.

With clinical examination, we found that the right tonsil presented a reddish-purple change in the central part of the tonsil, and in the bottom half, which persisted for some time, and did not spread to the palatine arches and soft palate (Figure 1). On the left side of the neck there were few lymph nodes with the size 8x6mm.



Figure 1. Black and violet change in the central part of the tonsil and in the lower half

Computerized tomography and magnetic resonance imaging of the endocranium, showed bilateral multiple metastatic hemorrhagic changes (Figure 2). Computerized tomography of the abdomen showed that in the lodge of the left adrenal gland persisted inhomogeneous expansive change of non homogeneous appearance, of mixed density, with diameter up to 70 mm. This tumor on the left performed impression and suppression on the medial contour of the left kidney, and on the right adrenal lodge also there is a hyperdense tumor formation, which can originate from an enlarged adrenal gland- secondary deposit (Figure 3).



Figure 2. Computerized tomography shows bilateral multiple endocranial hemorrhagic metastatic changes



Figure 3. Tumors in both adrenal lodges

The liver also presented a secondary deposit (Figure 4). Computerized tomographic examination of the thorax showed both sides of the lung parenchyma with occasional micronodular focal metastasis type changes of secondary deposit in diameter up to 5 mm (Figure 5).



Figure 4. Secondary deposits in the liver



Figure 5. Computerized tomographic examination of the thorax: shows in both sides of the lung parenchyma occasional micronodular focal changes of secondary deposit in diameter up to 5 mm

Biopsy and histopathological verification of the changes in the tonsil were performed and these confirmed the diagnosis (Figure 6, 7). Chemotherapy was arranged at another medical oncology clinic. Two months later the patient died.



Figure 6. Atypical melanocytes, mitosis and visible nucleoli. Abundant pigment in the cytoplasm HE x400



Figure 7. Small groups of tumor cells with pigment in the cytoplasm HE x 40

Discussion

Melanomas often arise from moles, dysplastic moles and to a lesser extent directly from the skin. Primary mucosal melanoma of the head and neck is an uncommon lesion and accounts for about 8% of all melanomas occurring in this region (5, 6). On chromosomes 9 and 11 defects, which are responsible for the initiation of melanoma, were identified, and under the influence of ultraviolet radiation comes to tumor progression. A group of Swiss scientists discovered a new gene mutation MEK1 and MEK2 (7), which affect the development of melanoma.

Melanomas can occur at any age, are rare in young children, and are somewhat more common in women in any part of the body skin. The disease course depends from the appearance and selection of aggressive tumor cells and capabilities of defense of the organism. Metastasis is mainly via lymphatic vessels (8), which are characterized by a gradual sequential. Hematogenous metastasis is possible but rare and occurs randomly in any organ. Regional lymph nodes are the most common site of metastasis, which means also satellite and accompanying metastasis.

There are microscopic metastases in the clinically unchanged lymph nodes in melanomas 1mm thick. Most commonly it metastasizes to the lungs, skin, liver, brain, kidneys and bones. Metastatic melanomas in the oral cavity, originating from distant sites are rarely seen (9). Most of melanoma metastasis can be detected on the basis of the appearance of symptoms or physical examination (10). Most patients die from disseminated disease that affects multiple organ sites of respiratory or cerebral complications. About 20% of patients with localized disease and 65% of node positive patients have asymptomatic distant metastasis at diagnosis.

In the literature are presented less than 30 cases of metastatic melanoma in the tonsils (11, 12), and our case is one of the few that has been added to this statistic.

As palatinal tonsils do not have afferent lymphatics, metastasis in this area is hematogenous. Nearly 60% of all skin melanoma metastasis demonstrate mucosa metastasis every 24 months. Some authors (13, 14) who state that the time for metastasis in palatinal tonsil ranges from 4 to 84 months after resection of the primary lesion. The literature describes that after the diagnosis of oral metastasis survival is usually about 2 months (15, 16).

Conclusion

Metastasis of melanoma in the oral cavity is rare. Melanoma metastasize through lymphatic and vascular routes. While it is expected that the regional lymph nodes are the first involved a complex anastomotic route between lymph channels may lead to metastasis to distant lymph nodes and organs, often in the nasal cavity, tonsils, tongue, nasopharynx, larynx and mouth, regardless of the location of the primary tumor. Metastasis to the mucosa of the upper aerodigestive tract shows a wide spread of melanoma with poor prognosis.

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Infliximab fails in a patient with solitary rectal ulcer syndrome

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Abstract

Solitary rectal ulcer syndrome (SRUS) is a rare disorder of defaecation characterized by bleeding per rectum, the passage of mucus and rectal pain. Current treatment includes laxatives, sucralfate, sulphasalazine, bowel retraining and surgery. However, these therapies have some limitations, and the effects cannot meet patient's demand. In this paper, we reported a patient presenting with recurrent periumbilical pain, increased stool frequency and tenesmus. Colonoscopy demonstrated that the intestinal mucous membrane showed congestion, edema and ulceration in the rectum. Except intestinal infectious, autoimmune, vascular, neoplastic diseases, final diagnosis was given as SRUS. Due to the ineffective therapy of glucocorticoid and sulfasalazine, infliximab was given intravenously at weeks 0, 2 and 6. However, the patient did not achieve the clinical remission after three times of infliximab infusion.

Key Words: Solitary rectal ulcer syndrome, infliximab, diarrhea, efficiency.

Introduction

Solitary rectal ulcer syndrome (SRUS) is a rare benign rectal condition that has a wide spectrum of a combination of symptoms, endoscopic findings, and histological abnormalities⁽¹⁾. It was initially described in 1829, while the first extensive publication occurred in 1969⁽²⁾. Clinical features include bleeding per rectum, the passage of mucus, prolonged excessive straining, perineal pain, feeling of incomplete empty, and sometimes rectal prolapsed⁽³⁾. Diagnosis can usually be made on colonoscopy. The histological examination of the rectal lesion is another key to the diagnosis of SRUS. Surface serration, fibromuscular obliteration, and crypt distortion consist of the characteristic features⁽⁴⁾. Topical treatments including salicylate, sulfasalazine, steroid, and sucralfate have no obvious efficacy to be found in adults⁽⁵⁾.

Tumor necrosis factor alpha (TNF- α) inhibitors (e.g., infliximab, IFX) have been widely used as an effective treatment for Crohn's disease and ulcerative colitis, and shown to induce mucosal healing, reduce steroid dependency or the risk for surgery or hospitalization, and improve the patient's quality of life^(6,7). In this study, we reported here a case with SRUS who received treatment with IFX. After IFX administration, the patient had persistent diarrhea on follow-up and colonoscopy revealed no changes of mucosal ulceration, suggesting a failure with IFX in the treatment of disease.

Case report

A 23-year-old male had recurrent belly pain for 6 years prior to consultation at the Department of Gastroenterology, Shanghai Tenth People's Hospital of Tongji University (Shanghai, China) on March 24th 2010. He also presented with diarrhea, increasing stool frequency (8-10 times per day), passage of mucous and rectal tenesmus. On this occasion, the patient was admitted to our hospital for further examination. Colonoscopy revealed 0.5×1.5cm sized, a well-demarcated longitudinal ulcer, with three polyps of about 1.0cm diameter in the rectum. The lesions were located at the distance of 6cm from the anal verge (Figure 1A). Mucosal biopsies were also taken and histological examination demonstrated a thickening of the mucosa with elongation and distortion of the glands (Figure 1B). Endoscopic ultrasonography showed thickening of the submucosa at the distance of 5cm from the anal verge (Figure 1C). Further laboratory examination was performed including routine blood and urine investigations, stool tests (microscopy and culture) for infections (bacterium, virus, parasites), autoantibodies, ASCA, p-ANCA, anti-TB antibody, food allergen, immunoglobulin, cytomegalovirus, Epstein-Barr virus, gonococcus, herpes simplex virus, mycoplasma, HIV, and results did not show any abnormal. Gastroscopy, chest radiography, electrocardiogram were normal. Small intestine spiral computerized tomography (CT) and abdominal CT angiography were also normal which proved to be negative for small intestine diseases and vascular diseases. Except other infectious, autoimmune, vascular, neoplastic diseases, the diagnosis of SRUS was made according to the symptoms and laboratory examination. The patient was treated with intestinal probiotics including bifidobacterium, lactobacillus acidophilus, enterococcus, enemas and 5-aminosalicylate suppository for two months (Salofalk, Losan Phama GmbH, Germany). Despite treatment the patient remained still symptomatic like diarrhea, passage of mucous and rectal tenesmus. With the informed consent of patients, IFX (Remicade; Cilag AG, Schaffhausen, Switzerland) was administrated at a dose of 5mg/kg intravenously at weeks 0, 2, and 6 to induce mucosal healing. Colonoscopy was then performed ten weeks after the first time of IFX injection, and the lesions in rectum did not come to any remission (Figure 2). The symptoms were totally persistent, and this therapeutic approach did not achieve the goal.



Figure 2. Endoscopical finding at week 10 after IFX administration

Discussion

SRUS is a rare benign disease, and the incidence of SRUS has been reported to be one in 100,000 per year in a 10-year study in Northern Ireland⁽⁸⁾. The exact etiology is not clear, one hypothesis proposes that trauma and ischemic damage to prolapsed mucosa due to excessive straining may play a role in the pathogenesis⁽⁹⁾, and another theory points to abnormal rectal evacuation due to paradoxical contraction of the puborectalis muscle. Moreover, abnormal defecation due to a reversed pressure gradient produced by the external anal sphincter may also be involved in the pathogenesis^(10,11).

The diagnosis of SRUS is based on symptomatology in combination with the endoscopic and histological findings. Symptoms include rectal bleeding, excess rectal mucus, constipation, tene-



Figure 1. Photographs were taken before treatment. A, Endoscopical finding at the beginning of therapy. B, Histological analysis shows irregular thichening of muscularis mucosa (original magnification $\times 200$). C, Thickening of the submucosa by endoscopic ultrasonography.

smus and incomplete defecation. The endoscopic appearance ranges from single to multiple ulcers, sometimes associated with patches of polypoid, hyperemic mucosa⁽¹²⁾. Lesions vary in size, number, and rectal wall position⁽¹³⁾. In previous reports^(13,14), the term SRUS is ambiguous and undoubtedly misnaming, as less than one-third of the endoscopic findings showed a single lesion. The majority of histological features include fibromuscular obliteration of the lamina propria, hypertrophied muscularis mucosa with extension of muscle fibres upward between the crypts, and glandular crypt abnormalities^(2,15). Consequently, because of the wide spectrum of clinical, endoscopic, and histological features, SRUS can be a great mimicks of other rectal disorders such as carcinoma and inflammatory bowel disease.

However, current treatments are suboptimal. In spite of correct diagnosis, outcomes can be unsatisfactory, with symptom and rectal lesion persistence. Chong et al.⁽¹⁶⁾ investigated some patients whose types of treatment locally available include bulking agents (Normacol, Metamucil and Lactulose), enemas (Steroid such as Predsol enema and Mesalamine), oral 5-aminosalicylate (5-ASA), endoscopical steroid injection and surgery. Eighty percent (12/15) of patients with enema treatment still had intermittent BPR on follow-up. Six of these patients had also been treated with steroid intra-lesional injection. Another study has shown that symptomatic improvement with bulk laxatives and bowel retraining was only seen in 19% of the patients⁽¹⁴⁾. There are no controlled trials of therapy but many patients improve, either spontaneously or with management of constipation⁽¹²⁾.

Anti-TNF agents (e.g. IFX) were first introduced in the 1990s. From then on, they achieved an important breakthrough in controlling Crohn's disease and ulcerative colitis. IFX is a recombinant immunoglobulin G1 kappa chimeric monoclonal antibody that potently and specifically binds to and neutralizes the soluble TNF- α homotrimer and its membrane-bound precursor⁽¹⁷⁾. Vos et al.⁽¹⁸⁾ observed a significant induction of regulatory macrophages in patients with mucosal healing after treatment with IFX. In HCT116 colonic epithelial cells, classically activated macrophage did not induce wound healing above the level of control. In contrast, alternative activated macrophage presented a good capacity to enhance wound healing up to 2-fold compared to classically activated macrophage or medium alone. In this study, IFX was found to be failure in the treatment of patient with SRUS. The pathogenesis is likely to vary in different patients. We propose that the patient is likely due to paradoxical contraction of muscle and forces on the rectal mucosa. The rectal pressures are increased, leading to ischaemia and ulceration. Consequently, IFX may play little role in the management of SRUS.

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Abstract

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Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

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