Original Article

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The Effect of Serum Bone Sialoprotein Levels on Tympanosclerosis

Serum Kemik Sialoprotein Seviyelerinin Timpanoskleroza Etkisi

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ABSTRACT

Objective: This study aimed to investigate serum bone sialoprotein (BSP) levels in patients with tympanosclerosis (TS).

Methods: We included into this study 24 patients with TS and 24 asymptomatic healthy volunteers. Our study consisted of two groups: TS group (n=24), comprising of patients who underwent surgery for chronic otitis media and had tympanosclerotic plaques; and a control group (n=24), comprising of healthy individuals without ear problems. The serum BSP levels were measured and the results were compared between the two groups.

Results: In the TS group, there were 15 female and nine male patients. The ages of the females ranged from 24 to 54 years, while those of the males ranged from 34 to 55 years; the average age was 40.46±9.75 and 43.11±6.77, respectively. In the control group, there were 14 females and ten males. The ages of the females ranged from 30 to 48 years, while those of the males ranged from 27 to 47 years; the average age was 40.25±5.28 and 38.4±27.08, respectively. There was a statistically significant difference in BSP levels between the two groups (p=0.001). In addition, there was a moderate positive correlation between BSP and hearing levels.

Conclusion: The serum BSP levels were higher in the TS group than in the control group and might have some effect on TS. Further studies on a large number of subjects using serum and tissue BSP levels should be designed to affirm the effects of BSP on TS.

Keywords: Timpanosclerosis, bone sialoprotein, calcification

ÖZ

Amaç: Bu çalışmanın amacı timpanoskleroz (TS) hastalarında serum bone sialoprotein (BSP) seviyelerini araştırmaktır.

Yöntemler: Yirmi dört TS hastası ve 24 asemptomatik sağlıklı gönüllü çalışmaya dahil edildi. Çalışmamızda iki grup vardı: kronik otitis media ameliyatı geçirmiş ve timpanosklerotik plakları olan hastalardan oluşan TS grubu (24); ve kulak problemi olmayan sağlıklı bireylerden oluşan kontrol grubu (n=24). Serum BSP seviyeleri ölçüldü ve sonuçlar iki grup arasında karşılaştırıldı.

Bulgular: Timpanoskleroz grubunda 15 kadın ve 9 erkek hasta vardı. Kadınların yaşları 24 ile 54 arasında değişirken, erkeklerin yaşları 34 ile 55 arasında değişmekteydi ve yaş ortalaması sırasıyla 40,46±9,75 ve 43,11±6,77 idi. Kontrol grubunda 14 kadın ve 10 erkek vardı. Kadınların yaşları 30 ile 48 arasında değişirken, erkeklerin yaşları 27 ile 47 arasında değişmekteydi ve yaş ortalaması sırasıyla 40,25±5,28 ve 38,4±27,08 olarak saptandı. BSP düzeyleri karşılaştırıldığında iki grup arasında istatistiksel olarak anlamlı fark bulundu (p=0,001). Ek olarak, BSP ile işitme seviyeleri arasında pozitif orta düzeyde bir korelasyon vardı.

Sonuç: Serum BSP düzeyleri TS grubunda kontrol grubundan daha yüksekti ve TS üzerinde bir etkisi olabilir. Çok sayıda hasta üzerinde serum ve doku BSP seviyelerini araştıran yeni çalışmalar, BSP'nin TS üzerindeki etkilerini doğrulamak için tasarlanmalıdır.

Anahtar Sözcükler: Timpanoskleroz, kemik sialoprotein, kalsifikasyon

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Introduction

Tympanosclerosis (TS) is a chronic middle ear disease that manifests as collagen accumulation in the tympanic membrane lamina propria and mastoid cavity submucosa and also affecting auditory ossicles (1). This disease involves the development of calcified plaques in the submucosal area of the middle ear, due to calcification of the connective tissue layer pathologically. Regarding the situation of calcification, we can observe by electron microscopy a network of intense collagen fibers with scattered crystalline material, which is fundamentally calcium phosphate. It is thought that the calcium phosphate clusters are formed by deposition of the mineral on the surface of matrix vesicles, which acts as primary sites of calcification (1,2). These changes often disrupt the movement of the eardrum and the ossicular chain, leading to hearing loss.

Although the etiology of TS is still not fully understood, it is widely believed that it commonly develops secondary to acute or chronic inflammation of the middle ear and as a result of other factors such as myringotomy, ventilation tube insertion, physical trauma, various chemical agent exposure, genetic predispositions, immunological processes and hypercalcemia (3).

Bone sialoprotein (BSP) is one of the most important extracellular matrix (ECM) proteins of the bone and belongs to the small integrin-binding ligand N-linked glycoprotein family. This family consists of BSP, dentin sialophosphoprotein, osteopontin (OPN), matrix extracellular phosphoglycoprotein and dentin matrix protein-1 that are believed to play crucial biological roles in the turnover, improvement and mineralization of dentin and bone (4,5). Although BSP is usually expressed in mineralized tissues and has been proposed to have a direct role in mineralization, the protein has currently been shown to be expressed in varied tissues including renal tissues, salivary glands and in pathological conditions such as tumours (6-8).

OPN is a hormone that is a regulator of biomineralization and inflammation and is also known as BSP I. Several studies have shown that OPN is upregulated in pathological ectopic calcification and tympanosclerotic focusing regions (9,10). Although the relationship between osteopontin and TS is known, the relationship between BSP and TS has not been investigated before. In this study, the serum BSP levels were evaluated in TS patients.

Methods

Following approval by the local ethics committee (Protocol number: 110, Date: 27.06.2018), the study was conducted in the Uskudar State Hospital Otolaryngology Clinic. Twenty-four patients who were diagnosed with chronic otitis media between July 2018 and March 2019 were included in the study. Patients with otorrhea, otitis media with effusion, acute otitis media, and chronic systemic disease were excluded. There were 48 participants: groups of 24 patients and 24 controls. The control group had no ear problems and no chronic systemic disease. There were 15 females and nine males in the patient group and the mean age was 40.46±9.75 and 43.11±6.77 years, respectively. There were 14 females and ten males in the control group and the mean age was 40.25±5.28 and 38.4±27.08, respectively (Table 1).

Sampling

Serum Bone Sialoprotein Levels

Serum BSP levels were evaluated using with a human enzyme-linked immunosorbent assay kit (lot no.: AK0018JAN10047; Elabscience, Wuhan, Hubei, PRC) and a Multiskan plate reader. (Thermo Scientific, Waltham, MA, USA). Anti-Human BSP antibodies in pre-covered 96-well plates were used. The biotin-conjugated anti-Human BSP antibodies were used as detection antibodies. Test samples, biotin-conjugate detection antibodies and standards were washed with the wash buffer after addition to wells. The samples and standards were analysed in duplicates as demonstrated in the kit package suffix.

Horseradish peroxidase (HRP)-Streptavidin was added to the unconjugated conjugates and further washed with washing buffer. TMB (tetramethylbenzidine) was catalysed with HRP to generate a blue colour product that turned yellow when the acidic stop solution was added. The quantity of Human BSP captured on the plate is proportional to the intensity of the yellow colour. The absorbances were read at 450 nm in a microplate reader and then the Human BSP concentrations were calculated according to the standard curve. The other serum parameters were examined using a Beckman Coulter AU 2700 Device (California, USA) and the spectrophotometric method.

Calibration was done with seven standards in the kit. The standards at 40, 20, 10, 5, 2.5, 1.25 and 0.63 ng/mL concentrations were ran twice and the standard curve was created. The standard curve was obtained in a similar manner to the shape recommended in the kit package insert. A well was separated as a blank well.

Biochemical Analysis: Total BSP (ng/mL) levels were measured from the serum samples of the patients.

The blood of the patients and the control group were centrifuged (10 min at 2,500 × g, 4 °C). Serum samples were stored in Eppendorf tubes at −80 °C until tested.

Statistical Analysis

In this study, IBM SPSS Statistics Version 22 software package was used for the statistical analysis. Shapiro–Wilks test was used for the normal distribution suitability of the parameters. Descriptive statistical methods (Mean, standard deviations and median values) were calculated. Mann–Whitney U test was used in the comparison of nonparametric data between groups. Significance was assessed at $p<0.05$ level. Spearman's correlation test was used in the correlation test performed between the hearing level and BSP level.

Results

The right side air and bone hearing levels of the patient group were 45.7±20.37 dB and 19.04±11.29 dB and the left side air and bone hearing levels were 40.54±21.69 dB and 17.54±12 dB, respectively (Table 1).

The right side air and bone hearing levels of the control group were 12.59±5.01 dB and 9.86±4.27 dB and left side air, and bone hearing levels were 13.31±5.42 dB and 9.77±4.51 dB, respectively (Table 1).

The mean serum BSP levels of the patient and control groups were 28.8±4.4 (ng/mL) and 24.1±4.9 (ng/mL), respectively. Statistical analysis of the patient and control groups revealed that serum BSP levels were significantly higher in the patient group compared to the control group ($p \leq 0.001$) (Table 2).

There was a moderate positive correlation between BSP and severity of hearing loss in the patient group ($r=0.49$; $r^2=0.319$; $p=0.01$) (Graphic 1).

Discussion

TS is a disease characterised by hyaline calcareous plaques in the tympanic membrane and tympanic space. It may be located in the ossicular chain, ligaments and sometimes in the mastoid. It is an irreversible disease that occurs as a sequelae of a poor inflammatory process in the case of a long-lasting chronic otitis media (11). The inflammatory state occurring in the middle ear mucosa progresses towards an abnormal scarring process characterised by hyalinisation, calcification and osteogenesis. These changes often disrupt the movement of the eardrum and ossicular chain, leading to hearing loss (12).

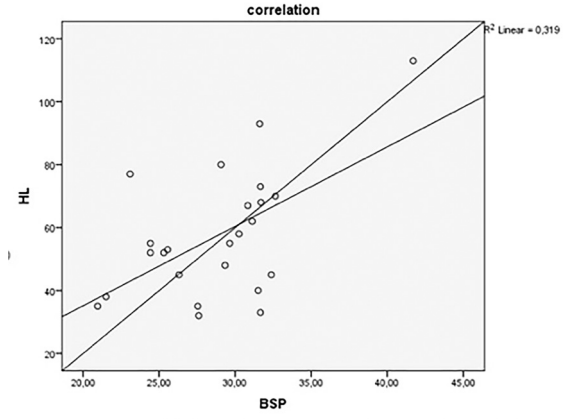
Table 1. Demographic data of the patient and control groups

Patient group	N	min - max	Mean ± SD
Female age	15	24-54	40.46±9.75
Male age	9	34-55	43,11 ± 6,77
Right air	24	10-87 dB	45.7±20.37 dB
Right bone	24	2-57 dB	19.04±11.29 dB
Left air	24	7-73 dB	40.54±21.69 dB
Left bone	24	0-50 dB	17.54±12 dB
Control group	N	min - max	Mean ± SD
Female age	14	30-48	40.25±5.28
Male age	10	27-47	38.4±27.08
Right air	24	3-20 dB	12.59±5.01 dB
Right bone	24	2-20 dB	9.86±4.27 dB
Left air	24	2-20 dB	13.31±5.42 dB
Left bone	24	2-17 dB	9.7±4.51 dB

N: Number of patients, SD: Standard deviation, dB: Decibel, min: Minimum, Max: Maximum

BSP is produced by osteoclasts, chondrocytes and osteoblasts and it is an important ECM protein (13-15). It was suggested to be physiologically significant for hydroxyapatite nucleation and mineralization (16,17). Although BSP does not contain different structural areas, the protein is highly modular, comprising spatially segmented motifs that can bind varied distinct ECM components with various biological roles, including collagen, matrix metalloproteinases, hydroxyapatite, as well as integrins present on many cell types (18-21). BSP might have a versatile act in the progress of the mineralized tissue. Spatiotemporal expression of BSP in *de novo* mineralization areas correlated with BSP at the start of mineralization (14,22). In addition, BSP is expressed at pathological areas of mineralization such as microcalcifications in the breast, atherosclerotic plaques, prostate and thyroid neoplasms (8,23-25). It is known that the basic pathology in tympanosclerotic tissues is excessive calcified hyaline plaques and pathological mineralization. There is no previous study in the literature investigating BSP levels in patients with TS. In our study, serum BSP levels were remarkably higher in the TS group than in the control group.

BSP is a highly post-translationally modified acidic phosphoprotein normally expressed in mineralized tissues such as dentin and bone (26). BSP was suggested to be physiologically important for hydroxyapatite nucleation, cell cohesion and collagen binding (16,17). BSP gene ablated mice with various skeletal and dental defects had decreased bone length and decreased cortical density (27,28). BSP gene-ablated mice also showed a delay in bone repair in cortical injury models; bone formation and absorption were also found to be impaired in bone marrow ablation models (29,30). Cementum is a mineralized tissue in the



Graphic 1. BSP: Bone sialoprotein, HL: Hearing level (moderate positive correlation between BSP and HL)

Table 2. Comparison of serum bone sialoprotein levels between patient and control groups

	N	Min	Max	Mean ± SD	p
Patient	24	20.96 (ng/mL)	41.7 (ng/mL)	28.8±4.4 (ng/mL)	0.001
Control	24	6.01 (ng/mL)	29.62 (ng/mL)	24.1±4.9 (ng/mL)	

Mann-Whitney U test $p \leq 0.05$, SD: Standard deviation, ng: Nanogram

cervical part of the root of the tooth. In immunohistochemical studies, a significant reduction in cementum accumulation was observed in BSP gene ablation mice (31).

BSP has been involved in the reparation of several mineralized tissues. Bone formation was observed when BSP and collagen implantation were performed in 7-8-week old rats with calvarial deficiency (32). Cortical default drilled into the femurs of BSP-ablated mice gets well slowly when compared to controls (33).

In a study comparing patients with metabolic bone disease and a healthy control group, serum BSP concentrations were found to be remarkably higher in patients with the metabolic disease. The highest concentrations were found in patients with active Paget's disease and secondary renal hyperparathyroidism (34).

In some studies, it has been suggested that osteoblast cultures can interfere with osteoblast binding through the selection of low-expressing clones or by the addition of an anti-BSP and that the antibody may have a negative effect on osteoblast distinction by changing BSP levels. This condition is presumably due to the breaking down of osteoblast differentiation (35-38).

In our study, we compared the serum BSP levels between the patient and the control group because we think that BSP plays an active role in tissue mineralization and calcification. In this study, serum BSP levels were significantly higher in the TS group than in the control group and there was a moderate positive correlation between BSP and hearing levels. The limitations of our study were that the number of patients was low and the amount of BSP at the tissue level was not measured.

Conclusion

The serum BSP levels were significantly higher in the TS group than in the control group. There was a moderate positive correlation between BSP and hearing levels. In the future, the manipulation of local BSP levels may be useful in the treatment of TS.

Ethics

Ethics Committee Approval: Following approval by the Local Ethics Committee (Protocol number: 110, Date: 27.06.2018), the study was conducted in the Uskudar State Hospital Otolaryngology Clinic.

Informed Consent: Written informed consent was obtained from the patients.

Peer-review: Internally and externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: A.B., Concept: A.B., Design: A.B., Data Collection or Processing: A.B., Ö.F.Ö., H.G.Ç., Analysis or Interpretation: A.B., M.Y., Ö.F.Ö., H.G.Ç., Literature Search: A.B., M.Y., Writing: A.B., M.Y.

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