Evaluation of Serum Fetuin-A, Parathyroid Hormone and Electrolyte Levels in Patients with Tympanosclerosis

Timpanoskleroz Hastalarında Serum Fetuin-A, Paratiroid Hormon ve Elektrolit Düzeylerinin Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to investigate serum fetuin-A, parathyroid hormone (PTH), and electrolyte levels in patients with tympanosclerosis (TS).

Material and Methods: Twenty-one patients with TS and 21 asymptomatic healthy volunteers were included in the study. There were two groups in our study: a TS group (n=21), comprising patients who underwent surgery for chronic otitis media and had tympanosclerotic plaques; and a control group (n=21), comprising healthy individuals without ear problems. The electrolyte, PTH, and fetuin-A levels in the serum were measured, and the results were compared between the two groups.

Results: Twenty-one patients (12 women, nine men) aged between 28 and 47 (average 38.6), and 21 volunteers (11 women, ten men) aged between 27 to 49 (average 38.8), were included in the study. When the levels of calcium and phosphorus were compared, no statistically significant difference was found between the groups (p=0.23, p=0.82). There was no statistically significant difference in serum fetuin-A and PTH levels between the patient and control groups (p=0.36, p=0.28). There was a moderate positive correlation in the correlation test between the level of hearing and serum fetuin-A (R value: 0.66).

Conclusion: The low serum fetuin-A levels in patients with tympanosclerosis may have an effect on the pathophysiology of the disease, but these observations were not statistically significant. Further studies on large number of subjects using serum and tissue Fetuin-A levels should be designed to affirm the effects of Fetuin-A on TS.

Key Words: Tympanosclerosis, Fetuin-A, Calcification

ÖZ

Amaç: Bu çalışmanın amacı timpanoskleroz (TS) hastalarında serum fetuin-A, paratiroid hormon (PTH) ve elektrolit düzeylerini araştırmaktır.

Gereç ve Yöntemler: Çalışmaya Yirmi bir TS hastası ve 21 asemptomatik sağlıklı gönüllü dahil edildi. Çalışmamızda iki grup vardı: kronik otitis media ameliyatı geçirmiş ve timpanosklerotik plakları olan hastaları içeren TS grubu (n = 21); ve kulak problemi olmayan sağlıklı bireyleri içeren kontrol grubu (n = 21). Serumdaki elektrolit, PTH ve fetuin-A düzeyleri ölçüldü ve sonuçlar iki grup arasında karşılaştırıldı.

Bulgular: Çalışmaya yaşları 28 ile 47 (ortalama 38.6±2.3) arasında değişen 21 hasta (12 kadın, 9 erkek) ile 27-49 arasında (ortalama 38.8±1.8) değişen 21 sağlıklı kontrol grubu (11 kadın, 10 erkek) dahil edildi. Serum kalsiyum ve fosfor düzeyleri karşılaştırıldığında gruplar arasında istatistiksel olarak anlamlı bir fark bulunmadı. (P=0.23, P=0.82) Serum fetuin-A ve PTH düzeyleri bakımından gruplar arasında istatistiksel olarak anlamlı farklılık saptanmadı. (P=0.36, P=0.28). İşitme seviyeleri ile serum fetuin-A düzeyleri arasında orta düzeyde pozitif korelasyon saptandı. (R:0.66).

Sonuç: Timpanosklerozu olan hastalarda düşük serum fetuin-A seviyeleri hastalığın patofizyolojisi üzerinde etkili olabilir, ancak bu gözlemler istatistiksel olarak anlamlı değildi. Fetuin-A'nın TS üzerindeki etkilerini doğrulamak için serum ve doku Fetuin-A seviyelerini kullanan çok sayıda denek üzerinde ileri çalışmalar tasarlanmalıdır.

Anahtar Sözcükler: Timpanoskleroz, Fetuin-A, Kalsifikasyon

INTRODUCTION

Tympanosclerosis (TS) is a chronic middle ear disease that manifests as collagen accumulation in the tympanic membrane lamina propria and mastoid cavity submucosa, also affecting the auditory ossicles (1). Pathologically, calcified plaques develop in the submucosa of the middle ear as a result of calcification of the connective tissue layer. Evaluation of the calcification with electron microscopy reveals a dense network of collagen fibers with interspersed crystalline material, which is mainly calcium phosphate. It is thought that the calcium (Ca) phosphate aggregates are formed by deposition of the mineral on the surface of matrix vesicles, which acts as primary sites of calcification (1-3). 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 TS 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, exposure to various chemical agents, genetic predispositions, immunological processes and hypercalcemia (1,4-9).

Fetuin-A is a glycoprotein that binds Ca and phosphate minerals and mediates the formation of a more stable and more soluble fetuin mineral complex in tissues and body fluids (10-12). In animal experiments, diffuse calcification was observed in all tissues as a result of a defect of the gene related to fetuin-A (10,13). This study aims to investigate serum fetuin-A, parathyroid hormone (PTH) and electrolyte levels in patients with TS.

MATERIALS and METHODS

The study included patients seen between 2014 and 2018 who were operated for chronic otitis and diagnosed with tympanosclerosis during the operation. The study included 21 patients who underwent surgery for chronic otitis media and 21 healthy volunteers. Exclusion criteria were otorrhoea within the previous 3 months, otitis media with effusion, ventilation tube insertion, otitis media with cholesteatoma, granulation tissue or polyps in the middle ear, myringosclerosis, and chronic systemic disease. Patients who had atherosclerosis, hypertension, altered liver and kidney functions, diabetes mellitus hyperparathyroidism, infectious diseases, elevated inflammatory indicators, history of drug use in the last 2 months, as well as those who were pregnant, lactating, children, elderly or obese (body mass index (BMI) > 24 kg/m^2), were also excluded from the study. Patients were divided into 2 groups (TS and control groups). The TS group (n=21) comprised patients who underwent surgery for chronic otitis media and had tympanosclerotic plaques on the middle-ear mucosa, ossicular chain or mastoid bone. The control group (n=21) comprised healthy individuals without ear problems or a known chronic systemic disease.

Sampling

Serum Fetuin-A and Electrolyte Levels:

Serum fetuin-A levels were evalauted using a human enzyme-linked immunosorbent assay (ELISA) kit (lot no.: AK0018JAN10047; Lab science, Wuhan, Hubei, PRC) and a Multiskan plate reader. (Thermo Scientific, Waltham, MA, USA). Anti-Human Fetuin-A antibodies pre-coated 96-well plates were used. Biotin-conjugated anti-Human Fetuin-A antibodies were used as detection antibodies. Test samples, biotin-conjugate detection antibodies, and standards were washed in wash buffer after addition to wells. HRP-Streptavidin was added to the unconjugated conjugates and further washed with washing buffer. TMB was catalyzed with HRP to generate a blue color product that turned yellow when the acidic stop solution was added. The quantity of Human Fetuin-A captured on the plate is proportional to the yellow density. The absorbances were read at 450 nm in a microplate reader, and then the Human Fetuin-A concentrations were calculated according to the standard curve. The other serum parameters were evaluated with a Beckman Coulter AU 2700 Device (California, USA) and the spectrophotometric method.

Biochemical Analysis: Total Ca (mg/dL), phosphorus (P) (mg/dL), fetuin-A (ng/mL) and PTH (pg/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 x g, 4°C). Serum samples were kept in Eppendorf tubes in the freezer at -80 °C until tested.

Statistical Analysis

The IBM SPSS Statistics Version 22 (IBM Turkish limited company, Istanbul, Turkey) program was used for statistical analysis. The normal distribution suitability of the parameters was evaluated by the Shapiro-Wilk test. Descriptive statistical methods (Mean, standard deviations, and median value) were calculated. The Mann-Whitney U test was used in the comparison of nonparametric data between groups. Spearman's correlation test was used in the correlation test performed between the hearing level and the fetuin-A level. Significance was assessed at $p<0.05$ level.

RESULTS

Twenty-one patients (12 women, 9 men) aged between 28 and 47 (mean 38.6), and 21 volunteers (11 women, 10 men) aged between 27 and 49 (mean 38.8), were included in the study.

The age of the male patients in the study ranged from 27 to 47 years and the mean age was 38.1±6.7 years. There was no statistically significant difference between the ages of the males in both groups (p=0.89).

The ages of the women in the study ranged from 28 to 48 years, and the mean age was 38.7±6.4 years. There was no statistically significant difference between the ages of the women in both groups (p=0.4).

The right and left air and bone values were 43.76±22.49, 16.47±14.62, 45.52±24.84 and 17.52±16.31 dB respectively in the TS group and 12.61±5.13, 9.95±4.8, 13.38±5.55 and 9.76±4.62 dB respectively in the normal group.

The data obtained from both groups are shown in Table I. There was no statistically significant difference in serum Ca and P levels between the 2 groups (p=0.23,0.82) (Table I).

There was no statistically significant difference in serum fetuin-A and PTH levels between the 2 groups (p=0.36, p=0.28) (Table I).

There was a moderate positive correlation in the correlation test between the level of hearing and serum fetuin-A. R value: 0.66 (Figure 1).

DISCUSSION

Although TS is usually seen between the ages of 30 and 50 years, it can be seen at any age (14,15). The pathogenesis of TS is not clear. The clinical observations indicate that there are some differences in the disease process between old and young patients. Tympanosclerosis is mostly restricted to the tympanic membrane in children but it can also affect the ossicular chain and mastoid cavity in adults. These changes are often accompanied by perforation of the tympanic membrane, varying degrees of destruction of the ossicles, and accompanying diseases such as hypercalcemia, hyperlipidemia or hypertension, and atherosclerosis (16).

Physiologically Ca levels depend on absorbed and excreted Ca. Persistent hypercalcemia, particularly with normal or elevated serum phosphate levels, can cause accumulation of ectopic Ca and phosphate in the walls of blood vessels, heart valves, cornea, and kidney parenchyma (9).

A diet rich in Ca can also cause an increase in TS formation. This may be seen with or without hypercalcemia (17). There was no statistically significant difference in blood Ca and P levels between the two groups in our study.

The main pathogenic features of TS are revascularization, fibroblast proliferation and accumulation of calcium deposits. Ca is required for sclerotic plaque formation, including the release of growth factors, cell migration, and cell proliferation. It is also potentially important for the secretion of collagen (18-20). Changes which affect Ca and P levels in the extracellular fluid may prevent pathological calcification and mineralization. Histopathologically, tympanosclerosis is similar to atherosclerosis, glomerulosclerosis and systemic calcinosis.

The macrophage might play a major role in the pathogenesis of TS. The microscopic components of sclerotic plaques are formed by calcifying microspheres (matrix vesicles) and are generally regarded as calcification residues or previous inflammation particles. During the acute phase of inflammation, macrophages are considered to be a

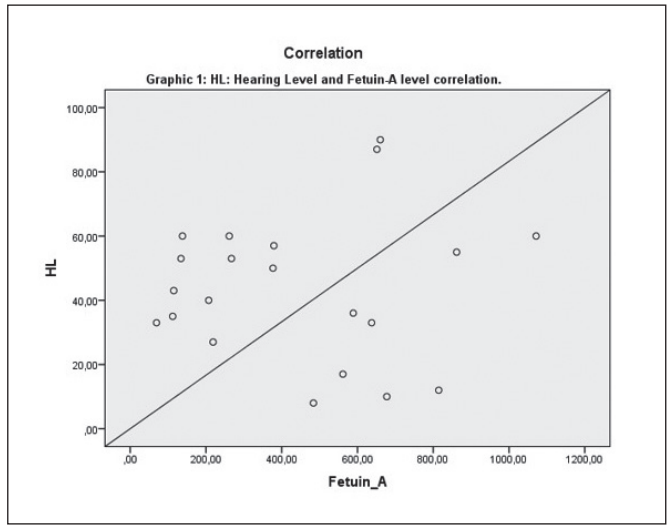

Figure 1: HL and Fetuin-A level correlation (Moderate levels of correlation present, R value: 0.66).

Table I: Comparison of patient and healthy control groups: Serum Fetuin-A, Parathyroid hormone, Calcium and Phosphorus.

Serum	Patients (n=21)	Control (n=21)	*p* value
Fetuin-A (ng/mL)	442.26±287.38	519.07±249.14	0.36*
Parathyroid hormone (pg/mL)	62.31±17.05	55.32±20.64	0.28*
Calcium (mg/dL)	9.57±0.36	9.7±0.35	0.23*
Phosphorus (mg/dL)	3.66±0.81	3.6±0.64	0.82*

Mann Whitney U Test, *p<0.05, Values are presented as mean ± SD. SD: Standard deviation

Ca phosphate particle collector. In the case of advanced or chronic inflammation and microsphere calcification, the capacity of the macrophages to clear calcium may be overcome. This results in the accumulation of calcium in the tissues and the formation of sclerotic plaques (21).

The parathyroid hormone (PTH) is a linear peptide comprising 84 amino acids secreted by parathyroid gland cells, which are mainly implicated in the regulation of the calcium-phosphorus metabolism (22). Several studies have shown that the effect of PTH overexpression on cardiovascular disease, including carotid artery, abdominal aortic, valvular calcifications and coronary artery calcification, while most of these studies focused on patients with renal failure, which is known to induce secondary hyperparathyroidism (23-25). In our study, there was no statistically significant difference in serum PTH levels between the patients with tympanosclerosis and the control group.

The molecules that inhibit the calcification process can be beneficial in the prevention of the TS process. Fetuin-A, expressed in the liver, is an important systemic calcification inhibitor with a high affinity for Ca and phosphate (26, 27). The main function of Fetuin-A is to prevent the crystallization of hydroxyapatite, and it regulates the calcification of tissue by temporarily forming soluble colloidal spheres containing Ca and phosphate (28).

In studies to investigate the pathophysiology of calcification in different tissue types, Fetuin-A has been shown to inhibit calcification in vascular smooth muscle cells (VSMCs) and osteoblasts (29,30). Addition of cellular-derived matrix vesicles from calcifying VSMC can accelerate calcification by inducing cell signaling changes and phenotypic alteration of recipient normal VSMC. The addition of cellular matrix vesicles with characteristics of low fetuin-A content enhanced the calcification of recipient VSMC (31). In this study, serum Fetuin-A levels were found to be lower in patients with tympanosclerosis compared to controls but this did not reach statistical significance level. The study of tissue Fetuin-A levels in tympanosclerosis patients may help explain the pathophysiology of the disease.

Low levels of Fetuin-A have been found to be associated with nephrocalcinosis, calcified coronary artery disease, basal ganglia calcification, nephrolithiasis and atherosclerotic calcification (32-36). However, the relationship between tympanosclerosis and serum fetuin-A levels has not been analyzed in the literature. In our study, no statistically significant difference was found between the groups regarding serum fetuin-A levels. Nevertheless, further studies on larger number of subjects using serum and tissue Fetuin-A levels should be designed to affirm the effects of Fetuin-A on TS.

CONCLUSION

This study suggests that the serum fetuin-A levels were lower in the TS group than the control group and might have some effect on TS, but these observations were not statistically significant. However, studies can be performed at the tissue level and in a larger number of subjects to confirm the effects of fetuin-A on TS.

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