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**Original Article / *Özgün Makale***

# Association between extensive tympanosclerosis and serum calcium, phosphorus, vitamin D, and parathyroid hormone levels

## *Yaygın timpanosklerozis ile serum kalsiyum, fosfor, D vitamini ve paratiroid hormonu düzeyleri arasındaki ilişki*

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***ABSTRACT***

**Objectives:** This study aims to investigate the association between extensive tympanosclerosis and serum levels of calcium, phosphorus, vitamin D, and parathyroid hormone.

**Patients and Methods:** This study was conducted in the Department of Otorhinolaryngology Head and Neck Surgery of the Kayseri Training and Research Hospital between October 2014 and May 2015. Thirty-seven patients who were diagnosed with extensive tympanosclerosis were assigned as a study group (18 males, 19 females; mean age 35.4 years; range 18 to 77 years). Thirty-two healthy subjects (15 males, 17 females; mean age 32.2 years; range 18 to 67 years) were recruited as a control group. Serum total calcium, phosphorus, vitamin D, parathyroid hormone and albumin levels were analyzed for each subject in the study. Total calcium levels were corrected based on the serum albumin levels to obtain albumin corrected calcium.

**Results:** While the mean values of serum phosphorus, vitamin D and parathyroid hormone were significantly higher in the study group, the difference was not significant for serum levels of total calcium and albumin corrected calcium between the two groups.

**Conclusion:** Although serum phosphorus, vitamin D and parathyroid hormone levels were found to be relevant for extensive tympanosclerosis in this study group, further studies are required to investigate the potential role of these serum parameters in cellular mechanisms of tympanosclerosis development.

***Keywords:*** Calcium; parathyroid hormone; phosphorus; tympanosclerosis; vitamin D.

***ÖZ***

**Amaç:** Bu çalışmada yaygın timpanosklerozis ile serum kalsiyum, fosfor, D vitamini ve paratiroid hormonu düzeyleri arasındaki ilişki araştırıldı.

**Hastalar ve Yöntemler:** Bu çalışma, Ekim 2014 - Mayıs 2015 tarihleri arasında, Kayseri Eğitim Araştırma Hastanesi Kulak Burun Boğaz ve Baş Boyun Cerrahisi Bölümü'nde yürütüldü. Yaygın timpanosklerozis tanısı konulan 37 hasta (18 erkek, 19 kadın; ort. yaş 35.4 yıl; dağılım 18-77 yıl) ile çalışma grubu oluşturuldu. Otuz iki sağlıklı birey (15 erkek, 17 kadın; ort. yaş 32.2 yıl; dağılım 18-67 yıl) kontrol grubu olarak alındı. Çalışmaya katılan her bireyin serum total kalsiyum, fosfor, D vitamini, paratiroid hormonu ve albümin düzeyleri analiz edildi. Serum albümin ile düzeltilmiş kalsiyum değerlerini elde etmek için, total kalsiyum seviyeleri serum albümin seviyelerine göre düzeltildi.

**Bulgular:** Ortalama serum fosfor, D vitamini ve paratiroid hormonu değerleri çalışma grubunda anlamlı oranda yüksek iken, serum total kalsiyum ve albümin ile düzeltilmiş kalsiyum değerleri bakımından iki grup arasında anlamlı fark yoktu.

**Sonuç:** Her ne kadar bu çalışma grubunda serum fosfor, D vitamini ve paratiroid hormonu düzeyleri ile yaygın timpanosklerozis arasında ilişki bulunsa da bu serum parametrelerinin, timpanosklerozis gelişiminin hücresel mekanizmalarındaki potansiyel rolü üzerine ileri çalışmalara ihtiyaç vardır.

***Anahtar sözcükler:*** Kalsiyum; paratiroid hormonu; fosfor; timpanosklerozis; D vitamini.

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Tympanosclerosis (TS) is a disease characterized by hyaline degeneration of the tympanic membrane and middle ear, followed by calcium and phosphorus depositions in the submucosa which appear as white, chalky patches.[1,2] Although the etiopathogenesis of TS has not been fully elucidated, it is widely accepted that it is a general and irreversible consequence of chronic inflammation.[3]

The inflammatory response in TS resembles that of atherosclerosis (AS). The incidence of TS was also found to be higher in patients with AS, which led researchers to investigate possible analogous risk factors.[4-6] Associations between serum levels of different parameters, including calcium, phosphorus, vitamin D and parathyroid hormone (PTH) and AS were previously described in several studies.[7-10] In the present study, we hypothesized that serum calcium, phosphorus, vitamin D and PTH levels can be associated with the presence of TS. Accordingly, we investigated these serum parameters in patients with extensive TS and compared them with healthy controls.

## PATIENTS AND METHODS

This study was approved by the local ethics committee (document no. 2014/26) and conducted in the Department of Otorhinolaryngology Head and Neck Surgery of the Kayseri Training and Research Hospital between October 2014 and May 2015. Written informed consent was obtained from the subjects and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Thirty-seven patients (18 males, 19 females; mean 35.4 years; range 18 to 77 years) who were diagnosed with extensive TS were assigned as the study group (SG). The diagnosis of TS was performed as described by Bluestone[11] and only patients with extensive TS involving both the tympanic membrane and middle ear with hearing loss were admitted to the SG. Otoscopic examination, temporal computed tomography evaluation and/or intraoperative observation findings were used to determine extensive TS. Thirty-two healthy subjects (15 males, 17 females; mean 32.2 years; range 18 to 67 years) were recruited as a control group (CG).

A detailed medical history, including audiological symptoms, was taken from the subjects of the study. All subjects underwent otoscopic examinations by a single examiner and conventional pure tone audiometry (0.25, 0.5, 1, 2, 4 and 8 kHz) was performed for each subject in the study in a standard soundproof room with a Grason-Stadler GSI-61 clinical audiometer (Madison, WI, USA). The mean of the four frequencies (0.5, 1, 2, 4 kHz) produced a pure-tone average (PTA) and air-bone gap (ABG) value. Air-bone gap was calculated as the difference between the air conduction-PTA and bone conduction-PTA thresholds. Subjects with diseases related to bone metabolism, liver or kidney failure, use of drugs affecting calcium metabolism, primary or secondary hyperparathyroidism, history of malignancy and osteoporosis and any history of otologic surgery were excluded from the study.

Venous blood samples were obtained from the subjects to determine laboratory parameters after overnight fasting. Serum total calcium (TC), phosphorus, vitamin D, PTH and albumin were analyzed for each subject in the study. Serum total calcium levels were corrected according to the serum albumin levels by the formula:[12] Albumin-corrected calcium (ACC)=TC+[0.8x(4-serum albumin concentration)].

Calcium, phosphorus and albumin values were evaluated photometrically in a chemistry autoanalyzer (AU680; Beckman Coulter Diagnostics, Miami, FL, USA) using original commercial kits. Parathyroid hormone values were measured using a chemiluminescent immunoassay on the UniCel DxI 800 Analyzer (Beckman Coulter Diagnostics, Miami, FL, USA). We used a sensitive liquid chromatography tandem mass spectrometry analytical method (The Thermo Scientific TSQ Quantum Access Max; Thermo Fisher Scientific, MA, USA) to detect serum vitamin D [25(OH)D] levels.

Statistical analysis was performed using SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). A two-tailed unpaired Student's t test was used to compare parametric conditions of the two groups and chi-square for comparison of categorical variables. Mann-Whitney U test was carried out for nonparametric conditions. A $p$ value less than 0.05 was considered significant for all comparisons.

## RESULTS

There was no statistically significant difference between the two groups with regard to age and sex ($p>0.05$). The mean values of PTA and ABG were 64.7±11.8 and 24.7±12.2, respectively, in the SG. The PTA values were within normal limits in the CG.

The mean values of serum TC, ACC, phosphorus, vitamin D, and PTH of the SG and CG are shown in Table 1. There was no significant difference between the two groups in terms of serum TC and ACC levels. On the other hand, serum phosphorus, vitamin D and PTH levels were significantly higher in the SG ($p<0.001$).

**Table 1**
Mean serum values of total calcium, albumin-corrected calcium, phosphorus, vitamin D, and parathyroid hormone in the study group and control group

| | Study group (n=37) | Control group (n=32) | |
|---|---|---|---|
| | Mean±SD | Mean±SD | *p* |
| Total calcium | 9.4±0.8 | 9.7±0.3 | 0.1 |
| ACC | 8.8±1.1 | 9.2±0.3 | 0.06 |
| Phosphorus | 4.3±0.7 | 3.2±0.5 | <0.001 |
| Vitamin D | 60.8±2.5 | 32.8±14.4 | <0.001 |
| PTH | 71.1±14.7 | 35.2±12.7 | <0.001 |

SD: Standard deviation; ACC: Albumin-corrected calcium; PTH: Parathyroid hormone.

## DISCUSSION

Although there are many studies concerning TS, the pathogenesis of TS is still poorly understood. Infection, trauma, genetic tendency, immunological reaction and *Helicobacter pylori* have been suggested as possible causative factors for TS[13-17] Because of the similar pathological mechanism between TS and AS, the incidence of TS was investigated in patients with AS. Koç and Uneri[14] found a high incidence rate of TS in atherosclerotic patients. Ferri et al.[4] reported that patients with carotid AS had a higher incidence of TS than control subjects. Following these results, a question was raised that gave a new direction for investigators concerning TS. Do TS and AS have any analogous risk factors contributing to the pathogenesis? Thus, some authors investigated well known causative risk factors of AS in patients with TS. Doluoglu et al.[5] reported that AS and TS were associated with identical risk factors, including high serum homocysteine, low-density lipoprotein, total cholesterol and triglyceride levels. Acar et al.[6] studied risk factors commonly accepted for AS in patients with TS and without TS and high homocysteine levels were found to be a risk factor for patients with TS. In the present study, we aimed to evaluate serum levels of TC, ACC, phosphorus, vitamin D and PTH in a selected patient group with extensive tympanosclerosis, which were previously described as risk factors for AS in several studies.[7-10]

Elevated levels of serum calcium and phosphorus were reported to be significantly associated with the presence of calcified atherosclerotic plaque.[8,9,18] In an experimental study, hypercalcemia induced by a calcium-rich diet was found to have an influence on the development of TS.[19] Calcium channel blockers were found to have a positive effect in preventing TS in rat animal models.[20,21] Caldas Neto et al.[22] reported that although myringosclerosis was found to be higher in patients with chronic renal failure than in control subjects, no influence of serum levels of calcium and phosphorus and PTH on the occurrence of myringosclerosis was determined in their study. In the present study, although the subjects in the SG consisted of patients with extensive TS, no significant relationship between serum TC and ACC levels and TS could be detected. On the other hand, serum phosphorus levels were found to be significantly higher in the SG than the CG, which may suggest that serum phosphorus levels may play a role in the development of TS, but not serum calcium levels. Shanahan et al.[23] reported that vascular calcification promoted by calcium can differ from that of phosphorus. In vascular smooth muscle cells, elevated phosphorus levels have a major role in osteochondrogenic differentiation, whereas elevated calcium levels promote predominantly cell apoptosis and vesicle release. The heterogeneity observed in the present study may have originated from different effects of calcium and phosphorus occurring at the cellular level in the pathogenesis of TS. Nevertheless, this speculation has to be investigated in further studies particularly focused on ultrastructural changes during the calcification process of TS.

The studies investigating a relationship between vitamin D and AS have produced conflicting results.[7] Among these studies, some investigators measured 1,25(OH)2D, the tightly regulated activated molecule of vitamin D, which can be within the normal range even in the presence of vitamin D deficiency.[24] In the present study, 25(OH)D, the primary circulating storage form of vitamin D, was preferred for measurement rather than 1,25(OH)2D. Because its half-life is longer than 1,25(OH)2D and its levels are closely influenced by the dietary intake of vitamin D,[24] 25(OH)D has been recommended as the best indicator of vitamin D status in individuals without chronic kidney disease. After careful evaluation of the literature, we found very few studies concerning an association between vitamin D and TS.[20,25] In an experimental study, Mann[25] reported that calcification in the TS process was prompted after vitamin D intoxication and simultaneous attic inoculation of *Streptococcus pyogenes*. In our study, we observed that serum vitamin D levels in the SG were significantly higher than those in the CG, which may suggest a possible effect of serum vitamin D on the TS process.

Higher PTH levels were found to be a causal factor for AS in both central and peripheral arteries.[10,26] Caldas Neto et al.[22] reported that serum levels of PTH did not have any effect on the occurrence of myringosclerosis

in patients with chronic renal failure. In contrast, in our study, serum PTH levels were significantly higher in patients with extensive TS than in healthy controls. This result may suggest that serum PTH has a causative role in the development of TS. Nevertheless, like the other parameters studied in the present study, serum PTH was measured only once and a single measurement may not reflect the alterations of these parameters during the development of TS. This constitutes a major limitation of our study.

In conclusion, although serum phosphorus, vitamin D and PTH levels were found to be relevant for extensive TS in the present study, further studies are required to investigate the potential role of these serum parameters in cellular mechanisms of TS development.

**Declaration of conflicting interests**

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