INTRODUCTION

Chronic otitis media (COM) is a chronic infection and inflammation of the middle ear. Although COM has been defined as a multifactorial disease, its etiopathogenesis has not been fully enlightened. Many factors, such as genetics, Eustachian tube disorder, autoimmune immunity, infection, osteoclastic activity, cytokines, endotoxins, and lipid peroxidation products due to oxidative stress, have been held responsible for the chronicity of inflammation in otitis media.

Cholesteatoma is a destructive squamous epithelial lesion of the temporal bone disrupting the balance between bone formation and resorption that gradually expands and leads to severe complications due to the destruction of nearby bony structures, ossicular chain, and otic capsule. Erosion in the ossicular chain and bony labyrinth may lead to hearing loss, vestibular dysfunction, facial paralysis, labyrinthine fistula, and intracranial complications. Although bone erosion can

OBJECTIVE: Sclerostin is a glycoprotein that plays a catabolic role in bone and is involved in the regulation of bone metabolism by increasing the osteoclastic bone resorption. In this study, serum sclerostin levels were measured in chronic otitis media (COM) with and without cholesteatoma, assuming that it might have a role in the aetiopathogenesis of bone resorption.

METHODS: A total of 44 patients with cholesteatomatous COM (cCOM) (n = 22) and non-cholesteatomatous COM (ncCOM) (n = 22) were included in this study, and 26 healthy volunteers without any chronic ear disease problem(s) constituted the control group (n = 26).

RESULTS: No significant difference was not found in terms of serum iPTH, ALP, and vitamin D levels between ncCOM, cCOM, and the control groups. A significant difference was found in terms of serum sclerostin, Ca, and P levels between ncCOM, cCOM, and the control groups (p<0.05). Serum sclerostin levels in the study groups were significantly higher but their serum Ca and P levels were significantly lower compared to the control group.

CONCLUSION: We think that serum sclerostin concentrations, which were significantly higher in patients with cCOM and ncCOM compared to healthy controls are associated with bone erosion. There is a need for further studies with larger samples in order to determine the relationship between sclerostin and bone erosion in cholesteatoma to help in establishing preventive measures against cholesteatoma and set new targets for the development of non-surgical treatments.

KEYWORDS: Otitis media. Cholesteatoma. Ear, middle. Bone morphogenetic proteins.

SUMMARY

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be observed in COM with and without cholesteatoma, it is more common in the cholesteatomatous type. Some studies that were carried out to investigate the aetiopathogenesis of bone resorption in cholesteatoma showed that cholesteatoma was associated with bone formation and absorption. There are also studies indicating that cholesteatoma is associated with other bone diseases such as osteoporosis. According to the majority of recent studies, osteoclasts act as “terminal function cells” in the bone resorption process. It has been demonstrated that osteoclasts exist in the ossicles eroded with cholesteatoma in the middle ear. The activation and maturation of osteoclasts play an essential role in bone resorption and remodeling of middle ear cholesteatoma. Nevertheless, the exact mechanism of bone resorption in cholesteatoma has not yet been explained.

Bone formation and resorption mechanisms are related to balanced functions of osteoblasts, and osteoclasts. Sclerostin is a glycoprotein that plays a catabolic role in the bone, increasing osteoclastic bone resorption by modulating the nuclear factor κB ligand: osteoprotegerin (rankl: opg) ratio in osteocytes, and it is involved in the regulation of bone metabolism. Sclerostin affects the activity of bone morphogenetic proteins (BMPs) and is an inhibitor of the metabolic pathway of wnt/β-catenin in bone cells. Osteocytes suppress the release of sclerostin in response to mechanical stimuli affecting the bone; thus, the osteogenic pathway promotes wnt/γ-catenin activation in osteoblasts. This signaling pathway plays an important role in osteogenesis and bone turnover. The antibodies directed against sclerostin are considered a new therapeutic option by increasing osteoblast-mediated bone formation while reducing osteoclast-dependent bone resorption in the treatment of osteoporosis with increased bone resorption and chronic inflammatory diseases, such as rheumatoid arthritis and ankylosing spondylitis.

The treatment of cholesteatoma is currently surgical; however, surgical treatment cannot compensate bone loss or prevent recurrence. There are a limited number of studies on the non-surgical treatment of cholesteatoma, and sclerostin antibodies may be considered a new therapeutic option in cCOM associated with bone erosion as well as in chronic inflammatory diseases such as rheumatoid arthritis and ankylosing spondylitis. There is no previous study on this subject in the literature.

The aim of this study was to investigate the aetiopathogenesis of bone resorption in COM by measuring serum sclerostin levels in patients with cCOM and ncCOM, help take preventive measures against the development of cholesteatoma, and set new targets for the development of non-surgical treatment strategies.

METHODS

A total of 70 participants consisting of 44 patients with cCOM (n=22) or ncCOM (n=22) were included in this prospective study, and 26 healthy volunteers without chronic disease with ear problems constituted the control group. The diagnosis of COM was made by anamnesis and otomicroscopic examination. All patients were examined by pure-tone audiometry and computed tomography (CT) of the temporal bone. The patients with soft tissue density in mastoid cells and the middle ear in CT were selected for the study. All patients underwent tympanomastoidectomy. The individuals aged below 15 years and those over 50 years, with diabetes mellitus, chronic kidney, and/or chronic liver diseases were excluded from the study. All individuals in the experimental and control groups were selected from the same geographical region, and blood samples were studied simultaneously. Blood samples were drawn in the morning after 12 hours of fasting before surgery. Blood samples were aliquoted by centrifugation at 1250 g for 10 minutes, and 1 mL aliquots of serum were collected and kept in the freezer (HERA Freeze, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at -80°C until the analysis. The samples were gradually brought to room temperature just before the analysis on the day of the study.

Serum Ca (Cat. No. 7D74-20), and P (Cat. No. 7D74-20) concentrations were measured with the Abbott kit and Architect c8000 instrument (Abbott, Chicago, IL, USA) by using standard procedures of the central laboratory of our hospital, serum iPTH (Cat. No. 8K25), and 1.25OH Vitamin D (Cat. No. 3L52) concentrations were measured with the Abbott kit and Architect i2000SR instrument (Abbott, Chicago, IL, USA).

Serum sclerostin concentrations were measured with an ELISA kit (Cloud-Clone Corp., Katy, TX 77494, USA). The intra-assay and inter-assay coefficients of variances (CVs) for sclerostin were less than 10% and <12%, respectively, with a measuring range of 0.312-20 ng/mL, and limit of detection of 0.118 ng/mL.

The participants who met the inclusion criteria were informed about the study and their approval
was received. Subsequently, they were included in the study. This study was carried out in adherence to the World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects after and ethical approval was obtained from the clinical research ethics committee of our institution under decision number of 2018/227.

Statistical analysis
For descriptive statistics, mean and standard deviation or median and minimum-maximum values were given in numerical variables, while the number was given as categorical variables. The normality hypothesis was tested by the Shapiro-Wilks test. In the comparison of groups, one-way analysis of variance (ANOVA) was used when assumptions for normality hypothesis were met, and the Kruskal-Wallis test was used when they were not met. In cases where there was a difference between the groups, paired comparison analyses were used to determine the group/groups that caused a difference. The results of analyses were evaluated using IBM SPSS v.21. The level of significance was set at p<0.05.

RESULTS
Demographic and clinical data of the ncCOM, cCOM, and control groups are presented in Table 1. No significant difference was found between the ncCOM, cCOM, and control groups in terms of female/male ratios (p=0.805) and mean age of the patients (p=0.970). No significant difference was found in terms of serum iPTH, ALP, and vitamin D levels between the patient and control groups. A significant difference was found in serum Ca levels between the patient and control groups (p<0.001). Serum Ca levels were significantly lower in the cCOM group compared to the control group (p<0.001) (Figure 1). A significant difference was found in serum P levels between the patient and the control groups (p=0.013). Serum P levels were significantly lower in the cCOM group compared to the control and ncCOM groups (p=0.026 and p=0.035, respectively) (Figure 2).

A significant difference was found in terms of serum sclerostin levels between the patient and control groups (p<0.001) (Table 2). Serum sclerostin concentrations were significantly higher in the cCOM and ncCOM groups compared to those in the control group (p<0.001 and p=0.001). The mean sclerostin plasma concentration was higher in the cCOM group in relation to the ncCOM patient group. However, this difference between the groups was not statistically significant (Figure 3).

DISCUSSION
This study demonstrated the relationship between the serum sclerostin levels of patients with ncCOM, cCOM, and healthy controls. How serum sclerostin levels were affected in patients with COM was investigated. The mean sclerostin plasma concentration in patients with COM was found to be significantly higher compared to age- and sex-matched healthy controls (p<0.001). When ncCOM and cCOM groups were compared, the mean plasma sclerostin concentration

| TABLE 1. DEMOGRAPHIC AND CLINICAL DATA OF THE PATIENT AND CONTROL GROUPS |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Gender, F/M                  | ncCOM                       | cCOM                        | Control                     |
|                             | 11/11                       | 9/13                        | 11/15                       |
| Age, year                   | 36.23 11.75                 | 37.05 11.0                  | 36.69 10.20                 |
| ALP, U/L                    | 68.41 14.28                 | 62.73 16.29                 | 67.92 18.01                 |
| Calcium, mg/dL              | 8.78 0.41                   | 8.48 0.41*                  | 9.04 0.48 <0.001           |
| Phosphorus, mg/dL           | 3.61 0.68b                  | 3.19 0.48b                  | 3.61 0.47 0.013            |
| iPTH, pg/mL                 | 53.35 [23 - 139]            | 49.35 [20.4 – 154.2]        | 40.85 [6.7 – 69.5]          |
| Vitamin D, ng/mL            | 8.65 [4.3 – 17.8]           | 91 [2.5 – 23.8]             | 11.65 [2 – 33.4]           |

* Significantly differs from control. b: Significantly differs from cCOM. The results are expressed as mean ± SD for the data with normal distribution, and median [min-max] for the data with non-parametric distribution

| TABLE 2. COMPARISON OF SERUM SCLEROSTIN LEVELS OF THE PATIENT AND CONTROL GROUPS |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Sclerostin, ng/mL           | ncCOM                       | cCOM                        | Control                     |
|                             | 3.77 1.31*                  | 3.89 1.22*                  | 2.55 0.61 <0.001            |

* Significantly differs from control
was higher in the group of patients with cCOM, without any statistically significant intergroup difference.

In a study carried out by Elemraid et al.\textsuperscript{10}, it was demonstrated that serum Ca values were lower in children with COM compared to healthy controls. Similarly, in a study carried out with adults in China, it was reported that people with normal serum Ca concentrations were less prone to COM.\textsuperscript{11} In our study, similar to the literature, while Ca was found to be significantly lower in the patients with cCOM and ncCOM compared to healthy controls, serum P levels were found to be lower in the cCOM group. Nevertheless, there was no significant intergroup difference for serum vitamin D, ALP, and iPTH values, which we interpreted as an indication of a lack of additional bone metabolic disease in our patients that could affect serum sclerostin levels in both groups.

The underlying mechanism of bone erosion in cCOM, which is a chronic inflammatory disease of the temporal bone, is still unclear. In the studies in the literature, the role of osteoclastic activity in bone resorption in cholesteatoma was emphasized. Firstly, in the ultrastructural study carried out by Chole et al.\textsuperscript{12}, evidence indicating that bone erosion was associated with osteoclastic activity in human and experimental cholesteatoma was found. Hamzei et al.\textsuperscript{13} reported that there were osteoclast precursor cells in all cholesteatoma tissue samples. Si et al.\textsuperscript{5} demonstrated the presence of osteoclasts in the bones eroded by middle ear cholesteatoma. Furthermore, Sudhoff et al.\textsuperscript{14} compared the cholesteatoma model in the temporal bone of mice with control samples, and they demonstrated significantly increased osteoclast density in affected mice. In a study carried out by Imai et al.\textsuperscript{15} in 2019, it was reported that there were more osteoclasts in the eroded bone adjacent to cholesteatomas than in unaffected areas. In our study, we found that sclerostin blood levels increased in cases with osteoclastic bone resorption in the cCOM group, which may lead us to infer that the increase in osteoclastic activity may be the result of the increased blood level of sclerostin. Contrary to the studies supporting the presence of osteoclastic activity in cholesteatoma, in some studies in the literature, it was reported that osteoclasts were not detected in the cholesteatomatous bones.\textsuperscript{16}

The studies indicating that cholesteatoma is associated with other bone diseases such as osteoporosis suggest that the osteoclastic activity in cholesteatoma may also lead to local bone erosion along with systemic bone erosion. In their cohort study, Wang et al.\textsuperscript{3}
found that the incidence of cholesteatoma increased in patients with osteoporosis. Individuals diagnosed with osteoporosis were excluded from the study so that our study results would not be affected. Thus, we aimed to investigate the effect of sclerostin on osteoclastic activity rather than osteoporosis.

Bone remodeling is a process involving bone resorption with osteoclasts and bone formation with osteoblasts.\(^7\) The studies on sclerostin, which affects bone remodeling and increases osteoclastic bone resorption by inhibiting the metabolic pathway of \(\text{wnt/}\beta\text{-catenin}\), have gained increasing interest in recent years.\(^18\) The fact that serum sclerostin levels in the cCOM group were found to be higher in our study supports the literature findings.

The formation of ncCOM and cCOM and the presence of inflammation coexistent with bone erosion are already known.\(^19,20\) It was demonstrated that osteoclastogenesis was activated by increased \(\text{RANKL}\) production due to inflammation in degenerative bone diseases, such as rheumatoid arthritis and periodontitis.\(^17,21\) Similarly, it was reported that osteoclastogenesis was induced by cholesterol-RANKL found in cholesteatomas.\(^15\) Sclerostin induces osteoclastogenesis through a pathway linked to \(\text{RANKL}\).\(^7\) The detection of higher serum sclerostin levels in the ncCOM and cCOM groups in our study supports the assumption that sclerostin may affect the development of cholesteatoma through inflammatory pathways.

The role of sclerostin in bone erosion observed in chronic inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis has been revealed in various studies. The level of sclerostin was found to be lower in patients with ankylosing spondylitis compared to healthy controls, suggesting the role of sclerostin in suppressing bone formation. Higher levels of sclerostin in patients with rheumatoid arthritis that may cause both local and systemic bone erosion compared to healthy controls have been associated with bone erosion. In our study, serum sclerostin levels were significantly higher in patients with ncCOM and cCOM compared to healthy controls. High levels of sclerostin indicate that bone erosion in ncCOM and cCOM may be associated with sclerostin. We haven’t encountered any study in the literature examining the relationship between cholesteatoma that can cause serious complications with bone erosion and serum sclerostin levels.

The established relationship revealed between sclerostin and bone resorption, as a potentially novel option in the treatment of diseases associated with bone erosion antibodies directed at sclerostin, has been investigated. As demonstrated in experimental animal models, the use of sclerostin antibodies in multiple bone diseases, such as myeloma and osteoporosis, decrease bone resorption.\(^22\) In the clinical studies carried out, it was demonstrated that the use of a sclerostin antibody in women with postmenopausal osteoporosis decreased bone resorption.\(^23\) Sclerostin antibodies, which are in the clinical development stage, can also be considered a promising option for the non-surgical treatment of cholesteatoma.

As a limitation of our study, we think that our detection of statistically insignificant levels of serum sclerostin in cCOM patients with higher bone erosion, in contrast to its higher levels in patients with ncCOM, was due to our small number of cases. There is a need for further studies with larger samples in order to determine the interaction between cholesteatoma and sclerostin. Furthermore, when the literature is reviewed, cases with hearing loss were observed in various disease groups, which may also be encountered in patients with cholesteatoma.\(^24-25\) The relationship between sclerostin level and hearing loss can also be evaluated in future studies.

**CONCLUSION**

We believe that serum sclerostin concentrations, which were significantly higher in patients with cCOM and ncCOM compared to healthy controls, are associated with bone erosion. The sclerostin levels that are increased both in inflammatory disorders and in osteoclastic hyperactivity may have a role in the progression of COM to cholesteatoma. There is a need for further studies with larger samples in order to determine the relationship between sclerostin and bone erosion in cholesteatoma since these studies may help to establish preventive measures against cholesteatoma and set new targets for the development of non-surgical treatment strategies.

This study was carried out in adherence to the World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects, after ethical approval was obtained from the clinical research ethics committee of our institution under decision number of 2018/227 at 29/11/2018. There are 272 words for the abstract and
RESUMO

OBJETIVO: A esclerostina é uma glicoproteína que desempenha um papel cataclítico no osso e também envolve a regulação do metabólismo ósseo, aumentando a reabsorção óssea osteoclástica. Neste estudo, os níveis séricos de esclerostina foram medidos em oitite média crônica (OMC) e com e sem colesteatomatoso, e presumiu-se-se que ela poderia ter um papel na etiopatogênese da reabsorção óssea.

MÉTODOS: Um total de 44 pacientes com oitite média crônica colesteatomatosa (OMCc) (n=22), não colesteatomatosa (OMCnc) (n=22) foram incluídos neste estudo, e 26 voluntários saudáveis e com doenças ósso-crânicas do ouvido constituíram o grupo de controle (n=26).

RESULTADOS: Não foi encontrada diferença significativa em termos de níveis séricos de iPTH, ALP e vitamina D entre OMCnc, OMCc e o grupo de controle. Foi encontrada uma diferença significativa em termos de níveis séricos de esclerostina, Ca e P entre OMCnc, OMCc e o grupo de controle (p<0,05). Os níveis séricos de esclerostina nos grupos de estudo foram significativamente mais altos, mas os níveis séricos de Ca e P foram significativamente mais baixos em comparação com o grupo de controle.

CONCLUSÃO: Acreditamos que as concentrações séricas de esclerostina, significativamente maiores em pacientes com OMCc e OMCnc em relação aos controles saudáveis, estão associadas à erosão óssea. Há necessidade de mais estudos com amostras maiores para determinar a relação entre esclerostina e erosão óssea no colesteatomato, já que essas pesquisas podem ajudar a estabelecer medidas preventivas contra o colesteatomato e novas metas para o desenvolvimento de tratamentos não cirúrgicos.

PALAVRAS-CHAVE: Oitite media, Esclerostina, Colesteatomato, Orelha Média.

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