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OTOLOGY

Association between occurrence of ossicular chain defect and osteoprotegerin gene expression in patients with chronic otitis media

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Abstract

Objective Chronic otitis media (COM) is an important debilitating public problem causing hearing loss due to irreversible resorption of the ossicular chain. Activation of osteoprotegerin (OPG) during an acute attack of COM prevents bone resorption.The aim of the study was to investigate the role of OPG gene expression level on ossicular chain resorption in chronic otitis media.

Materials and methods Fifty operated COM patients were included in the study. While 20 patients underwent ossiculoplasty, 30 patients underwent type 1 tympanoplasty. For RNA isolation and OPG gene expression analysis, middle ear swabs were taken from nasopharynx in the ostium of the Eustachian tube. RNA was isolated with mRNA easy kit and kept at − 85 °C till the cDNA and expression analysis. Expression levels were analyzed with real-time quantitative PCR in comparison with PDGB gene expression level as an internal control.

Results Sample Cq measurements of type 1 tympanoplasty group were higher than Cq measurements of the internal control group ($p = 0.027$; $p < 0.05$). In contrast, there was no statistically significant difference between sample Cq measurements of ossiculoplasty group and Cq measurements of the internal control group ($p = 0.293$; $p > 0.05$).

Conclusion Since OPG gene expression level was significantly higher in type 1 tympanoplasty group, OPG gene regulation system may have an effect on ossicular chain destruction in COM.

Keywords Tympanoplasty · Ossiculoplasty · Osteoprotegerin · Gene expression

Introduction

Chronic otitis media (COM) is a chronic infection and inflammation of the middle ear [1]. Osteoprotegerin, also known as tumor necrosis factor receptor superfamily member 11b (TNFRSF11B), is a protein inhibiting bone destruction caused by osteoclasts [2, 3].

COM usually results from recurrent attacks of acute otitis media or chronic otitis media with effusion [1]. Hearing can be affected due to ear drum perforation, effusion and ossicular chain distruction in COM. There are medical and/or surgical treatment options for patients with COM according to the stage of thedisease. The principal targets of treatments are first the eradication of the infection and second hearing restoration. In patients with ossicular chain damage, mechanism of the sound transmission is distorted and hearing loss develops. The most frequently affected ossicle is the long process of the incus. To provide restoration of hearing function, an intact tympanic membrane, a well-ventilated middle ear space lined with mucosa and ossicular chain which will provide sound transmission between the tympanic membrane and inner ear, should be constituted. Ossicular reconstruction may be performed in the same session with tympanoplasty and/or mastoidectomy or second look [4, 5].

Ossicular chain may be intact or eroded in COM without cholesteatoma. Middle ear structures may be destructed due to the duration of disease, the number of recurrent acute inflammatory attacks and age of onset [4]. The levels and

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structures of the genes effective on the inflammatory microenvironment of the middle ear may change associated with the frequency of inflammatory attacks. As recurrent inflammation causes permanent damage to each organ affected, it leads to bone resorption which is the most destructive damage in the sensitive organ with restricted volume like the middle ear. If the role of genes in the pathological process of COM is understood, new genetic treatment models may be the main topic for future researches. Gene therapy may be effective to recover inflamatuar effects and prevent ossicular damage. OPG is activated during an acute attack of inflammation and reduces bone destruction [2, 3]. In the literature, there are studies indicating that ossicular morphology is affected in COM, tympanosclerosis and advanced otosclerosis. Additionally, there are studies performed in the literature related to low level of OPG leading to a predisposition for bone destruction [6–13]. However, there is no study performed in the literature related to the association between OPG-RANKL system and development of ossicular chain defect in COM without cholesteatoma and, to the best of our knowledge, our study is the first one performed about this subject.

This study was aimed to investigate the role of inflammatory gene expression on ossicular chain resorption in COM patients who underwent type 1 tympanoplasty or ossiculoplasty.

Materials and methods

A total of operated 50 COM patients (17 males and 33 females) aged 15–62 years (38,79 ± 13,79) were included in the study. Surgery was performed in all patients. Types of surgeries were as follows: 20 ossiculoplasty (six Partial ossicular replacement prosthesis, six Total ossicular replacement prosthesis, eight applications of bone cement), 30 type 1 tympanoplasty. Before we start this study, power analysis was done. And G*Power (v3.1.7) programme was used to determine sample size. At the beginning of the study, ten persons were included in each group to do pilot study and when Cq difference values were evaluated, the extent of impact was calculated as $d = 0.92$. In addition, statistical analysis showed that ten persons should be in each group (total 40 person) to get 80% power at the level of $\alpha = 0.05$.

Approval was received from the local ethics committee before the study. Informed consent form was given to all patients and the requirements of the Declaration of Helsinki developed by the World Medical Association were followed.

Characteristics of the groups included in the study:

First group

Twenty patients with COM underwent ossiculoplasty. There was soft tissue density in the mastoid bone and middle ear at temporal bone computed tomography (CT) and ossicular chain defect was detected in the surgery.

Second group

Thirty patients with COM underwent type 1 tympanoplasty. There was no soft tissue density in the mastoid bone and middle ear at temporal bone CT. In addition a normal ossicular chain was detected in the surgery.

The patients with cholesteatoma, revision and tympanomastoidectomy cases were excluded from the study. One sample in tympanoplasty group was also excluded due to contamination.

RNA expression analysis

Within the scope of the project, the total RNA expression analysis study was performed on middle ear swabs taken from nasopharynx in the ostium of the Eustachian tube in operated patients with COM. QIAGEN RNA easy kit (Hilden, Germany) was used for gene expression study and Biotech Rabbit cDNA synthesis kit was used for RNA expression analysis study of samples. cDNA concentrations were measured using Thermo Fisher Nanodrop (Massachusetts, USA). OPG primers were added using 50 ng of cDNAs obtained for each sample and expression analysis was performed with real-time PCR including ROX dye. Primers required for PBGD which was internal control in the reaction were studied simultaneously in the same cDNA. Differences in the quantification cycle (Cq) values were compared and the results were evaluated.

Results

The results of the study are summarized in Table 1 and Fig. 1.

Evaluation of Cq measurements according to the groups

There was no statistically significant difference between sample Cq measurements of ossiculoplasty group and Cq measurements of the internal control group ($p = 0.293$; $p > 0.05$). Sample Cq measurements of type 1 tympanoplasty group were higher than Cq measurements of the

Table 1 Evaluation of Cq measurements according to the groups

	Ossiculoplasty group ($n=20$)	Type 1 tympanoplasty group ($n=29$)	p
Sample Cq			
Min/max (median)	21.7/35,6 (27,3)	12.9/39.6 (29.6)	
Mean ± SD	27.78 ± 3.54	28.29 ± 7.20	
Control (PBGD) Cq			
Min/max (median)	16.1/39.5 (30)	11.2/38.2 (25)	
Mean ± SD	29.46 ± 6.51	24.70 ± 8.40	
[c]p	0.293	0.027*	
Difference of Cq			
Min/max (median)	− 11.8/13.5 (− 3.5)	− 11.0/19.3 (4.1)	[b]0.042*
Mean ± SD	− 1.68 ± 6.94	3.59 ± 8.29	

[a]Student's t test

[b]Mann–Whitney U test

[c]Paired samples t test *$p<0.05$

Cq Quantification cycle, *PBGD* porphobilinogen deaminase

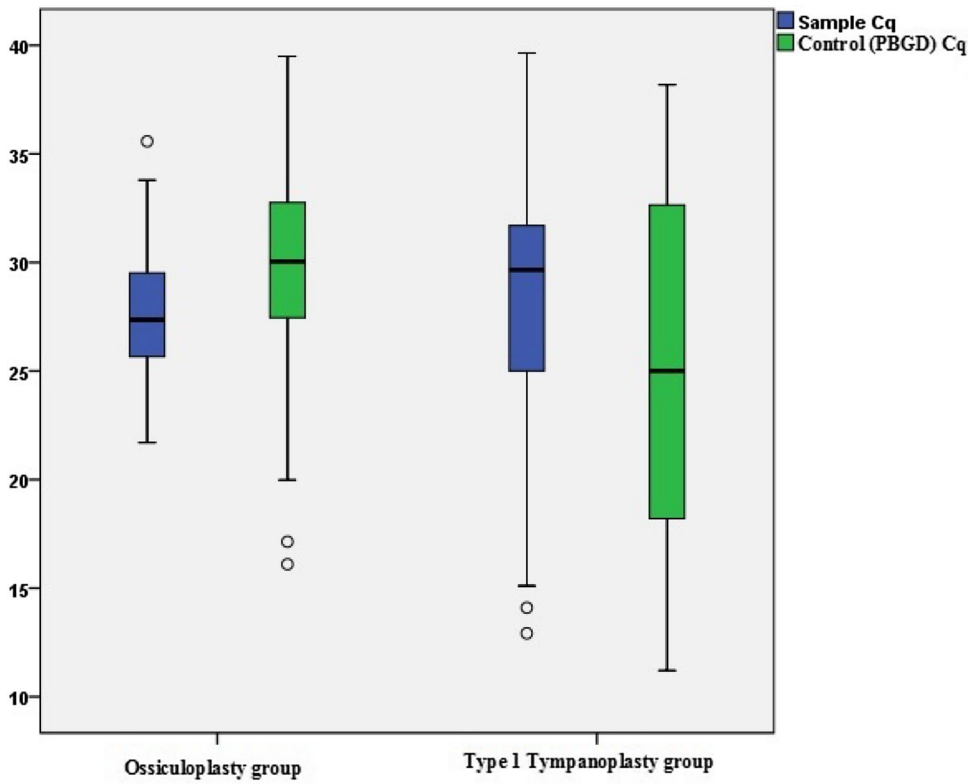

Fig. 1 Distribution of Cq measurements according to the groups

internal control group and this was found to be statistically significant ($p=0.027$; $p<0.05$).

Differences between sample Cq measurements and internal control Cq measurements were statistically significant according to the groups ($p=0.042$; $p<0.05$); the difference in the tympanoplasty group was higher than the difference in the ossiculoplasty group.

Evaluation of levels of difference of Cq measurements according to the groups

The results were grouped as high, medium and low expression levels (Differences of Cq measurement: 0–6 low; 6–10 medium and 10 high) according to the analysis of differences of Cq. Accordingly, distribution of 49 samples was

as follows: 10 samples with high expression level, 12 samples with medium expression level and 27 samples with low expression level. In the evaluation performed, it was determined that there were 7 samples with high expression level, 7 samples with medium expression level and 15 samples with low expression level in type 1 tympanoplasty group and 3 samples with high expression level, 5 samples with medium expression level and 12 samples with low expression level in ossiculoplasty group. There was no statistically significant difference between levels of the difference between sample Cq measurements and internal control Cq measurements according to the groups ($p = 0.799$; $p > 0.05$).

Statistical evaluations

In this study, NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for the statistical analysis. Student's *t* test was used for the intergroup comparisons of parameters with normal distribution and Mann–Whitney *U* test was used for the intergroup comparisons of parameters without normal distribution. Paired Samples t test was used for in-group comparisons of quantitative data with normal distribution. Fisher–Freeman–Halton exact test was used for comparison of qualitative data. Significance was evaluated at a level of $p < 0.05$.

Discussion

Ossicular morphology is affected in COM, tympanosclerosis and advanced otosclerosis. Previous studies showed that it was higher in COM patients with cholesteatoma [4, 5]. Chole et al. reported that while bone destruction was observed more frequently, especially in COM patients with cholesteatoma, it was seen also in COM patients without cholesteatoma [6]. On the other hand, in another study performed by Kurihara et al., while ossicular chain defect was 80% in COM patients with chloesteatoma, this rate was only 10–20% in patients with simple COM without cholesteatoma [7]. However, there is no objective explicit knowledge in the literature about the pathophysiology of ossicular chain defect in the chronic otitis media without cholesteatoma. It is well known that while ossicles are destructed in some cases, they are intact in other cases with COM without cholesteatoma. This condition brings the question of "Is there a difference between these two groups regarding a genetic predisposing factor triggering ossicular chain destruction?" to mind. The reply to this question has a place regarding the pathophysiology and approach for the treatment of the disease.

Osteoprotegerin also known as tumor necrosis factor receptor superfamily member 11b (TNFRSF11B) is a protein-inhibiting bone destruction caused by osteoclasts. In recent investigations, it has been determined that OPG secreted by osteoblasts and defined to be soluble receptor suppresses osteoclastogenesis by binding RANKL and plays an important role in bone metabolism [8]. In a study performed by Hofbauer et al., the authors found that serum OPG levels were reduced in multiple myeloma patients with lytic lesions [9]. Zehnder et al. demonstrated in their study performed in the mice that hearing was seriously affected in consequence of remodeling in the middle ear and inner ear due to reduced OPG level [10]. In the study performed by Whyte et al., the authors demonstrated that hearing was seriously affected due to reduced OPG level in the middle ear and inner ear in patients with juvenile Paget's disease developing due to increased RANK signaling resulting from suppressed OPG expression in consequence of mutation in OPG gene [11]. Zupan et al., collected human bone samples from 54 age and sex-matched patients with osteoporosis (OP) or osteoarthritis (OA) during hip arthroplasty surgery. The expression of 25 genes encoding pro-inflammatory cytokines, their receptors, osteoclast-specific genes and RANK/RANKL/OPG genes was measured using quantitative real-time PCR. They found that the relationship between osteoclastogenic and anti-osteoclastogenic pro-inflammatory cytokines differs in human OP and OA bone and could present an important factor for characteristics of OP and OA bone phenotypes. Although this study was related to hip joints, the study results were still important to show the relationship between bone/joint pathology and osteoclastogenic/anti-osteoclastogenic pro-inflammatory cytokines [12]. In a recent study, the expression of mRNA for osteoclast biomarkers and regulating factors in middle ear cholesteatomas was studied to elucidate the level of osteoclast activity in this disease. Bone powder from mastoidectomy was collected from 14 patients for analysis. This study indicated that osteoclasts were unlikely to be activated in cholesteatomas. However, the weakness of this study was low number of patient [13].

However, there is no study performed in the literature related to the association between OPG-RANKL system and development of ossicular chain defect in COM without cholesteatoma and, to the best of our knowledge, our study is the first one that investigates this subject. Besides that, while the levels of OPG protein was investigated in previous studies, in our study genetic expression differences which could provide a contribution to ossicular chain defect were investigated.

In our study, there is no significant difference between patients with and without ossicular chain defect in the group undergoing surgery in terms of the duration of disease. All of our patients are the ones treated with the surgical method without active infection and followed up with intact fascia.

In this study, difference in Cq value (difference between sample Cq and internal control Cq values) indicating expression level of OPG gene was higher in type 1 tympanoplasty

compared to ossiculoplasty($p=0.042$; $p<0.05$). At the same time, there was no significant difference between sample Cq and internal control Cq in the ossiculoplasty. In contrast, sample Cq was higher compared to internal control Cq in type 1 tympanoplasty ($p=0.027$; $p<0.05$). Since OPG has a preventive effect from bone destruction by inhibiting osteoclast activation assessed, this results mean that OPG osteoblastic activation was higher in type 1 tympanoplasty.

Conclusion

The difference between the two groups undergoing surgery in this study is the need for ossicular chain reconstruction due to ossicular chain destruction. On the other hand, both groups did not have cholesteatoma. In this study, the expression level of OPG gene was investigated to evaluate the presence of genetic basis playing a role in the management of osteoblast activation regarding the occurrence of ossicular chain defect.

The expression level of OPG gene which had a preventive effect from bone destruction by inhibiting osteoclast activation was found to be significantly higher in type 1 tympanoplastycompared to ossiculoplasty ($p=0.042$; $p<0.05$). The presence of a statistically significantly higher level of osteoblastic activity in type 1 tympanoplasty shows that OPG may have a preventive role in the pathogenesis of development of ossicular chain destruction. According to our opinion, new studies including a large number of patients and investigating the role of OPG in the occurrence of ossicular chain defect in COM are needed.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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