European Position Statement on Diagnosis, and Treatment of Meniere’s Disease

MAGNAN, Jacques, et al.

Abstract

Meniere Disease keeps challenges in its diagnosis and treatment since it was defined by Prosper Meniere at the beginning of the 19th Century. Several classifications and deﬁnition were made until now and speculations still exist on its etiology. As the etiology remains speculative the treatment models remain in discussion also. The European Academy of Otology and Neurotology Vertigo Guidelines Study Group intended to work on the diagnosis and treatment of Meniere’s disease and created the European Positional Statement Document also by resuming the consensus studies on it. The new techniques on diagnosis are emphasized as well as the treatment models for each stage of the disease are clarified by disregarding the dilemmas on its treatment. The conservative, noninvasive and invasive therapeutic models are highlighted.

Reference


PMID: 30256205
DOI: 10.5152/iao.2018.140818
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by Arianna Di Stadio, Massimo Ralli
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Editorial

A Big Step Forward

Scientific publishing is a challenging process. An institution's hard-to-achieve sequences demand that the editor is dedicated toward hard work. The scientific quality of the content is the main task while achieving this job and is the deciding factor on which eligibility for admission depends. The endeavor by scientists and researchers is to exhibit the outcome of their work and share it with the scientific community with great attention.

Currently, the scientific merit of a Journal relies on acceptability by indexing organizations. Prerequisites for being indexed are primarily based on publication ethics and eligibility. Conditions that are introduced by the indexing organizations become hard to achieve with an increase in the robustness of selection criteria.

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Audiovestibular Loss of Function Correlates in Vestibular Schwannomas

Niels West, Martin Nue Møller, Søren Hansen, Per Cayé-Thomasen

INTRODUCTION
Vestibular schwannomas (VSs) are benign tumors originating from the Schwann cells of the vestibular branch of the eighth cranial nerve. Recent studies have shown that the incidence of sporadic VS tumors is increasing steadily and is currently estimated to be approximately 20 cases per million/year [1]. Asymmetric sensorineural hearing loss and tinnitus are the most common symptoms, but vertigo is also frequently occurring, indicating the affection of vestibular system function. Despite being of vestibular nerve origin, magnetic resonance imaging (MRI) screening protocols for VS are based on hearing acuity rather than vestibular function [2]. Several studies have shown an association between VS and loss of vestibular function as evaluated by, for example, caloric response [3-6] and vestibular evoked myogenic potentials (VEMPs) [3, 5-8]. However, our knowledge remains limited regarding vestibular function in relation to tumor size and hearing acuity. In addition, little is known on tumor affection of the different parts of the vestibular system, as well as a potential relationship between the function of the individual parts of the vestibular system, i.e., the neuroepithelia of the saccule, utricle, and cristae of the semicircular canals.

Periodic MRI evaluations of tumor progression are based on size [9]. Further knowledge on alternative measures regarding tumor size and loss of audiovestibular function seems warranted.

The aim of the present study was to investigate the relationship between the tumor size and the outcomes of audiovestibular tests in patients with sporadic VS, as well as the potential relationships between the degree of hearing loss and the degree of vestibular function loss, as evaluated by a vestibular function test panel.

OBJECTIVES: The aim of the present study was to investigate the relationships between tumor size, hearing, and vestibular outcomes in patients with vestibular schwannomas (VSs).

MATERIALS and METHODS: Adult patients (n=124) with unilateral extrameatal VS prior to surgery were included in the study. This was a retrospective cohort study of preoperative audiovestibular investigations including audiometry, discrimination test, caloric test, cervical vestibular evoked myogenic potential (c-VEMP), and ocular vestibular evoked myogenic potential (o-VEMP).

RESULTS: The difference between lesioned and non-lesioned ear was significant for all audiovestibular outcomes. The mean caloric deficit was 74%. No tumor sided o-VEMPs were elicited. Caloric deficit correlated with hearing loss measured with pure tone average and discrimination score. c-VEMP deficit was significantly associated with severe hearing loss and larger tumors.

CONCLUSION: The presence of VS leads to a significant deterioration of audiovestibular function in all objective measures. Caloric test and o-VEMPS are sensitive though unspecific measures of VSs. Increasing tumor size is not directly associated with hearing loss and only somewhat to vestibular deficit. However, audiovestibular findings are correlated.

KEYWORDS: Hearing loss, vestibular disorders, acoustic neuroma, surgery, treatment, diagnostics

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MATERIALS AND METHODS

Data on tumor size, hearing, and vestibular function were retrieved retrospectively from the files of 127 consecutive patients operated at our tertiary referral center by the translabyrinthine (TLA) or retrolabyrinthine (RLA) approach for sporadic VS. Indication for surgery was either tumor size or tumor growth. The study was conducted in accordance with the Declaration of Helsinki. Informed consent and ethics committee approval were not applicable as the study was based on retrospective file review.

The tumor size was determined by the largest extrameatal tumor diameter on the latest preoperative MRI and classified according to the consensus on reporting VS size by Kanzaki et al. [10]. According to the system, the extrameatal part of the tumor can be small (1–10 mm), medium (11–20 mm), moderately large (21–30 mm), large (31–40 mm), and giant (>40 mm). Isolated intrameatal tumors have an extrameatal extension of 0 and comprise their own subgroup of tumors. However, to enable subgroup analysis of small tumors, the intrameatal tumor group and small tumor group were merged.

Hearing acuity was determined the day before surgery by pure tone audiometry and speech discrimination score (DS). The pure tone average (PTA) was determined by the average of the thresholds of 250, 500, 1000, 2000, 4000, and 8000 Hz.

The vestibular test panel (also performed the day before surgery) consisted of caloric test with bithermal irrigation and quantified according to slow phase velocities (Aqua Stim; Interacoustics, Middelfart, Denmark). Unilateral weakness (UW) was graded as defined by Tringali et al. [11] as UW <20% was considered normal, UW between 20% and 70% signified moderate hypofunction, and UW >70% implied severe hypofunction. Cervical vestibular evoked myogenic potential (c-VEMP) for test of saccular function and ocular vestibular evoked myogenic potential (o-VEMP) for test of utricular function consisted of air-conducted clicks of 100 dBnHL (decibel normal hearing level) presented consecutively to each ear (Eclipse; Interacoustics, Middelfart, Denmark). The VEMP results were assessed binarily to determine whether a potential was elicited or not. All vestibular tests were performed by the same investigator.

GraphPad Prism version 7.0 for Mac OS X (GraphPad Software, San Diego, CA, USA) was applied for data analyses [12]. Mann–Whitney tests for non-parametric data were performed to compare groups. Spearman coefficients ($r_s$) and chi-square ($\chi^2$) tests were calculated for correlation analysis. A p-value <0.05 was defined as significant.

RESULTS

Three tumors were excluded from the analysis because the tumor was found to belong to the facial nerve. All included tumors (n=124) were isolated intrameatal tumors (n=4) and tumors with extrameatal extension (n=120). The cohort consisted of 66 (53%) women and 58 (47%) men. Tumors were right-sided in 63 (51%) cases and left-sided in 61 (49%) cases. The mean age of the patients was 55 (standard deviation, SD 13.4) years at surgery. The mean extrameatal size of all tumors was 19 (SD 5.9) mm. Regarding size, 11 tumors were graded as small, 61 tumors were graded as medium, and 52 tumors were graded as moderately large.

Hearing Acuity

The mean DS on tumor side was 52% compared with 96% on the contralateral side, resulting in a mean DS difference of 44% between the two ears. Of the patients, 90% had an asymmetrical DS, of which 97% were worst on the tumor ear. The mean PTA were 55 (SD 27.1 dB) dBHL (decibel hearing loss) for tumor ears and 20 (SD 16.4 dB) dBHL for non-tumor ears (Table 1, Fig. 1). There were no significant differences in hearing acuity (PTA and DS) according to tumor grade (Table 2).

VESTIBULAR FUNCTION

All patients except two had vestibular hypofunction as evaluated by caloric response on the tumor ear. One patient had bilateral normal
response, and one patient had hypofunction to the non-tumor ear. The mean caloric hypofunction was 72%. Of the caloric findings, 95% were abnormal (>20% hyporeflexia) of which all were tumor sided. Of the c-VEMPs, 66% were asymmetrical. Of these, all but four represented negative c-VEMPs on the tumor ear and positive c-VEMPs on the contralateral ear. The remaining 34% had symmetrical c-VEMPs, either bilaterally positive or bilaterally negative. Of these, 22% elicited a c-VEMP response on the tumor ear compared with 81% on the healthy ears. Then, 37% of the o-VEMPs were asymmetrical, in all cases by a negative response on the tumor ear and a positive response on the non-tumor ear. The symmetrical o-VEMPs (67%) were all bilaterally negative. Thus, no tumor sided o-VEMPs were elicited (Fig. 2).

Correlations between Tests
Using Spearman coefficients, there was a strong correlation between PTA and DS \( (r_s=0.86, p<0.001) \). The correlations between PTA and DS against the caloric function were weak \( r_s=0.25 \) and \( r_s=0.27 \). There were no correlations between tumor size and audiometric tests. In addition, there was no correlation between tumor size and caloric function.

In the intrameatal and small tumor subgroup, 45% of the tumors elicited a c-VEMP potential compared with 14% in the medium tumor group \( (p=0.02) \) and 26% in the moderately large tumor group \( (p=0.19) \).

Patients with an absence of c-VEMPs showed higher PTA thresholds (two-tailed \( p=0.07 \) and one-tailed \( p=0.035 \)), though the same group did not have significantly lower DS. Similarly, there was no association to severe caloric deficit. Since all o-VEMPs were negative on the tumor side, tests of correlations between o-VEMPs and other tests were not possible.

Surgical Approach
There were significantly more small- and medium-sized tumors than larger tumors in the RLA group \( (\chi^2, p=0.02 \text{ and } p=0.003, \text{ respectively}) \). There was no difference in approach between small- and medium-sized tumors \( (p=0.82) \). The mean PTA and DS in the RLA group were 32 dBHL and 94% compared with 72 dBHL and 53% in the TLA group \( (p<0.0001 \text{ and } p<0.0001) \). The mean caloric hypofunction was UW=41% in the RLA group compared with UW=65% in the TLA group \( (p=0.001) \). There was no difference between surgical approach and elicitation of VEMPs \( (p=0.4) \).

DISCUSSION
Vestibular schwannomas are known to cause unilateral damage to both auditory and vestibular functions \[3, 5, 9, 13-15\]. For all individuals in the present study, there was a highly significant difference between lesioned and non-lesioned side for all auditory and vestibular tests. Our patient population represents a selected subgroup of patients with VS, since they have been elected for surgery because of accelerated tumor growth or symptom complaints. The difference between the ears is expected to be somewhat greater among this particular patient group than the background VS population that also counts smaller asymptomatic tumors \[9, 16\]. The mean tumor side PTA and DS corresponded to a class C hearing loss according to the American Academy of Otolaryngology–Head and Neck Surgery classification \[17\].

Almost all patients (98%) had a lesioned side caloric hypofunction. The mean UW of 72% corresponds to a severe hypofunction according to a previous classification \[11\].

There was absence of o-VEMPs on the tumor side with positive contralateral o-VEMPs in 37% of the cases, indicating superior vestibular nerve (SVN) affection on the lesioned side. Most tumors are located on the inferior vestibular nerve (IVN) and as a consequence affecting c-VEMP signals. The results indicate that SVN is affected in all cases of VS though most tumors originate from IVN \[3, 18\]. The aim for both c- and o-VEMP tests is to evaluate the corresponding end organ, which is the saccule and utricle, respectively. Data suggest that these organs appear to have different sensitivity for the presence of a tumor. However, these organs are supplied by afferents from different parts of the vestibulocochlear nerve. A recent histopathological study has documented that VS in one part of the nerve leads to loss of ganglions in the corresponding end organ, potentially leading to deteriorating function \[19\]. SVN and related blood supply are anatomically susceptible to entrapment due to the relationship to the internal
auditory canal wall \[20\]. Hearing loss could also be a result of external compression of the cochlear nerve. Anterograde degeneration of end organs may also be present \[19, 20\]. Our clinical results are in agreement with these observations, although specific perioperative tumor location (SVN or IVN origin) was not available. It seems impossible to predict tumor location on the basis of VEMPs alone, which is in accordance with other findings \[21\]. Being inherently sensitive to tumor, it may be possible to assemble a battery of audiovestibular tests that could indicate tumor growth, instead of the rising patient population enrolled for routine periodic MRI. In recent literature, the video head impulse test has proven effective in detecting peripheral vestibular disorders \[22\], including VSs \[23-25\]. However, the specific clinical value and correlation with other vestibular test findings need further investigation.

The positive correlation between the auditory tests was expected \[26-28\]. However, tumor size failed to correlate with PTA and DS. Previous histopathological studies have shown that >80% of the ganglions have to be lost before a shift in PTA threshold occurs, and that the loss of ganglions affects discrimination and PTA disproportionately \[29\]. Contrary to our results, other studies have shown a correlation between caloric hypofunction and size \[8, 11, 21\]. An explanation for the missing correlation in our study could be the relatively small size interval of tumors of which 91% had a diameter of 11–30 mm. This interval differs from the other studies that have a larger distribution of small tumors and a larger range of sizes \[8, 11\]. In the present study, the medium and moderately large groups were similarly affected on all audiovestibular parameters (Table 2), which could indicate a ceiling effect on the loss of function for many tumors in this size range.

There was an association between good hearing (PTA) and c-VEMP potential, indicating that pure tone hearing loss is associated with loss of vestibular function as measured with VEMP. However, the association between hearing and canal paresis was weak.

Canal paresis was more prevalent in the group lacking c-VEMP potential; however, the difference was not significant. As found in previous studies \[6, 30\], four patients without canal paresis was missing c-VEMP, which may be explained by the location of the tumor since c-VEMP and caloric test investigate the inferior and superior nerve pathways, respectively. Preserved c-VEMP was associated with smaller tumors, which is in agreement with other studies \[8, 25\].

The difference in PTA, DS, and size between the RLA and TLA groups was expected since surgical approach is determined by the presence of good hearing of smaller tumors \[14\]. It is interesting, however, that even though caloric deficit had only weak to non-existent association with hearing and size, the deficit was significantly lower in the RLA group. In contrary, VEMPs were independent of surgical approach.

A weakness of MRI in assessing VS size is the neglect of the intrameatal lesion that might cause vestibular deficits \[9, 10, 24\]. In addition, a limitation of the study is the varying time between the last MRI and the audiovestibular tests, the latter being performed the day before surgery. Thus, the true size of a tumor may be underestimated. These issues could explain the missing correlations between deficit and tumor size.

CONCLUSION

The present study correlates tumor size with audiovestibular tests by horizontal comparisons of individuals that may respond differently to tumors of the same size due to anatomical and physiological differences. The study finds a significant deterioration of audiovestibular function in the presence of VS in all objective measures. Both caloric test and VEMPs are sensitive yet unspecific measures regarding tumor side. In the present study, increasing tumor size has only a limited association with loss of peripheral vestibular function and has no significant effect on hearing loss.

Though inconsistently affected, audiovestibular function is highly sensitive to the presence of VSs; however, the interdependent factors between audiovestibular parameters remain to be fully understood. Future studies should appreciate a vertical perspective linking individual tumor progression and function.

REFERENCES


INTRODUCTION

The pathology underlying sudden sensorineural hearing loss, which is known as sudden deafness (SD), remains unknown. It affects the unilateral ear in most cases and is often accompanied by tinnitus and vertigo [1]. The hearing level can recover within the first 1 or 2 months, and after this period, the hearing level will be fixed. Several underlying pathologies, such as vascular disturbance and viral infection, are taken into consideration [2, 3].

SD is thought to have multiple causes, including genetic and environmental factors. To date, some gene polymorphisms have been identified for the vasculature- or inflammation-related pathogenesis of SD, such as protein kinase C-eta 1425G/A, matrix metalloproteinase-1 1607G/2G, methylenetetrahydrofolate reductase 677C/T, prothrombin 20210G/A, platelet Gly IIIaA1/A2, factor V Leiden 1691G/A, interleukin-1A-889C/T, interleukin-6C 572C/G, complement factor H 402Y/H, and nitric oxide synthase 3 894G/T.

OBJECTIVES: The pathology of sudden sensorineural hearing loss, which is known as sudden deafness (SD), remains unknown. The purpose of this study was to investigate the association between mitochondrial uncoupling protein 2 (UCP2) polymorphism and SD risk.

MATERIALS and METHODS: We compared 83 patients suffering from SD and 2048 controls who participated in the Longitudinal Study of Aging at the National Institute for Longevity Sciences. Multiple logistic regression was used to calculate the odds ratios (ORs) for SD with a polymorphism of the UCP2 (rs660339) gene.

RESULTS: Under the additive model of inheritance, UCP2 polymorphisms showed significant association with a SD risk. The OR was 1.468 (95% confidence interval, 1.056–2.040) with an adjustment for any past history, such as diabetes, dyslipidemia, or hypertension, and for age and sex.

CONCLUSION: Our results imply that the UCP2 (rs660339) polymorphism has a significant association with the risk of developing SD.

KEYWORDS: Genetic polymorphisms, sudden deafness, uncoupling protein 2

INTRODUCTION

The pathology underlying sudden sensorineural hearing loss, which is known as sudden deafness (SD), remains unknown. It affects the unilateral ear in most cases and is often accompanied by tinnitus and vertigo [1]. The hearing level can recover within the first 1 or 2 months, and after this period, the hearing level will be fixed. Several underlying pathologies, such as vascular disturbance and viral infection, are taken into consideration [2, 3].

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polymorphisms [14-18]. Environmental factors related to lifestyle, including short sleeping times, heavy smoking, alcohol abuse, and fatigue, have been found to be risk factors for SD [14, 15].

Uncoupling proteins (UCPs) are mitochondrial transporters located in the inner mitochondrial membrane and are able to uncouple adenosine triphosphate (ATP) from mitochondrial respiration. Among the five UCPs, UCP2 has a wide expression in almost all mammalian tissues, such as white adipose tissue, kidney tissue, and liver tissue [19]. The UCP2 mRNA transcripts have an abundant expression in the spiral and vestibular ganglia in the inner ear of rats [17, 18]. Sugiyama et al. [19] have described that the UCP2 Ala55Val polymorphism (rs660339) was significantly associated with age-related hearing loss. The objective of the present study was to investigate the association between UCP2 polymorphism and SD.

MATERIALS and METHODS

Patients

In total, 83 patients (39 males and 44 females; mean age, 58.0±14.2 years; range, 22–86 years) with SD were enrolled at the Nagoya University Hospital between November 2007 and March 2011, and a genetic analysis was performed on these patients. SD was defined as a type of deafness or hearing loss with an unknown etiology and with a sudden onset complicated by no cranial nerve palsy other than the auditory nerve. We used the criteria established by the Sudden Deafness Research Committee Study Group of the Ministry of Health and Welfare (1973), Japan.

Controls

The controls population were recruited from the Longitudinal Study of Aging at the National Institute for Longevity Sciences (NILS-LSA). Entrants in the NILS-LSA hospital were sampled at random from resident registrations, and they were stratified by age and sex. The area for the NILS-LSA study was located within approximately 30 km of the Nagoya University hospital. The details of the NILS-LSA have been described previously [20]. The participants responded to a series of surveys helpful in collecting population statistics and clinical data, such as comorbidities. Those with a history of SD in the surveys were excluded. In total, 2048 participants (1033 males and 1015 females; mean age, 59.2±10.9 years) were included as controls. These subjects completed the first examination of NILS-LSA between November 1997 and April 2000, and the analyses of UCP2 rs660339 gene were sampled.

Ethics

The study protocol was reviewed and approved by the ethics committees of Nagoya University (370-4) and by the National Center for Geriatrics and Gerontology (#14, #52, and #74), and written informed consent was obtained from all subjects.

Genotype Analysis

Genomic DNA was extracted from the lymphocytes isolated form peripheral blood using standard protocols, and polymerase chain reaction (PCR) amplification was performed. Genotyping using an allele-specific primer (ASP) method was also performed (Toyobo Gene Analysis, Tsuruga, Japan), as described previously [21]. The data of the primer sequences and PCR conditions are presented in Table 1.

Hearing Evaluation

We evaluated the hearing levels in patients with SD using the audimeter (Model AA-79S; Rion, Tokyo, Japan) in a silent chamber. The average hearing level was expressed as the average score recorded at five frequencies (250, 500, 1000, 2000, and 4000 Hz, respectively). The hearing level results of patients with SD were assessed against the criteria of the Ministry of Health and Welfare, Japan [22]. The recovery rate was divided into four classes: complete recovery (all frequencies on the final audiogram were ≤20 dB or improvement to the same degree of hearing level as in the other side of ear), remarkable improvement (improvement in the hearing level of ≥30 dB on an average), slight improvement (improvement in the hearing level of ≥10 dB but <30 dB on an average), and no change (improvement in the hearing level of <10 dB on an average). A good recovery comprises complete recovery and remarkable improvement. A poor recovery comprises slight improvement and no recovery. Because the possibility of recovery of hearing is extremely low at 1 month after the disease onset, to analyze the hearing recovery, SD patients who first visited our hospital within 1 month of disease onset were selected.

Statistical Analysis

Statistical analyses were conducted using the Statistical Analysis System software, version 9.1.3 (SAS institute, Cary, NC, USA), with significance achieved at a p value of <0.05. Univariate analyses of the categorical variables were performed using the chi-squared test. Student’s t test was used to evaluate the differences in the continuous variables between the two groups. Multiple logistic regression was performed to calculate the odds ratios (ORs) for SD risk concerning the UCP2 polymorphism for the multivariate analysis. Genotypes were defined as major allele homozygotes (CC), heterozygotes (CT), and minor allele homozygotes (TT) in UCP polymorphism. The major allele was determined for practical reasons.

We used the additive genetic model for the analyses, and the minor allele frequency was compared between patients with SD and controls by allocating scores of 0, 1, and 2 to major allele homozygotes, heterozygotes, and minor allele homozygotes, respectively. We used

Table 1. PCR condition used in genotyping the gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs No</th>
<th>Labeled primers</th>
<th>Sequence (5'→3')</th>
<th>Amplicon (F1/R)/(F2/R) (bp)</th>
<th>Annealing temp.(°C)</th>
<th>Mg (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP2</td>
<td>rs660339</td>
<td>F1 (FITC)</td>
<td>CCAGTGCGCGCTACAxCC</td>
<td>65/72</td>
<td>65</td>
<td>2.5</td>
</tr>
<tr>
<td> </td>
<td> </td>
<td>F2 (Texas Red)</td>
<td>CCAGTGCGCGCTACAxTC</td>
<td>55</td>
<td>67.5</td>
<td>4.5</td>
</tr>
<tr>
<td> </td>
<td> </td>
<td>R (Biotin)</td>
<td>TCAGAATGGTGCCCATACA</td>
<td> </td>
<td> </td>
<td> </td>
</tr>
</tbody>
</table>

PCR: polymerase chain reaction; ASP: allele-specific primer; UCP2: uncoupling protein 2; FITC: fluorescein isothiocyanate
Table 2. Characteristics of case and control groups

<table>
<thead>
<tr>
<th></th>
<th>SD group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>83 (3.9%)</td>
<td>2,048 (96.1%)</td>
<td></td>
</tr>
<tr>
<td>Sex, % male</td>
<td>47.0</td>
<td>50.4</td>
<td>0.5375</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.0±14.2</td>
<td>59.2±10.9</td>
<td>0.3365</td>
</tr>
</tbody>
</table>

SD: sudden deafness

Table 3. Genotype distribution in SD cases and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls Total n=2048</th>
<th>SD patients Total n=83</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>UCP2 (rs660339) CC</td>
<td>549 (26.81)</td>
<td>15 (18.07)</td>
<td>0.1571</td>
</tr>
<tr>
<td>CT</td>
<td>1032 (50.39)</td>
<td>44 (53.01)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>467 (22.80)</td>
<td>24 (28.92)</td>
<td></td>
</tr>
</tbody>
</table>

SD: sudden deafness; UCP2: uncoupling protein 2

Table 4. Risk of SD associated with the additive model

<table>
<thead>
<tr>
<th>Mode of inheritance</th>
<th>Crude: model 1</th>
<th>Adjusted: model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>UCP2 (rs660339)</td>
<td>1.352 (0.987–1.851)</td>
<td>1.468 (1.056–2.040)</td>
</tr>
</tbody>
</table>

SD: sudden deafness; UCP2: uncoupling protein 2; OR: odds ratio; CI: confidence interval

Table 5. Allele frequency in SD case with good and poor recovery

<table>
<thead>
<tr>
<th></th>
<th>SD cases with good recovery</th>
<th>SD cases with poor recovery</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>22</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>UCP2 (rs660339) C allele, T allele</td>
<td>20, 24</td>
<td>33, 43</td>
<td>0.8289</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.2±12.1</td>
<td>59.5±15.8</td>
<td>0.5701</td>
</tr>
<tr>
<td>Average hearing level (dB)</td>
<td>74.7±19.6</td>
<td>68.2±25.7</td>
<td>0.3071</td>
</tr>
<tr>
<td>Period from onset to first visit (days)</td>
<td>4.6±4.6</td>
<td>7.8±8.8</td>
<td>0.1178</td>
</tr>
</tbody>
</table>

SD: sudden deafness; UCP2: uncoupling protein 2

The results of multiple logistic regression are shown in Table 4. A significant difference in SD risk was observed between patients with SD and controls in terms of UCP2 (rs660339) polymorphisms following adjustments for age, sex, and diseases, and the OR for SD risk was 1.468 (95% confidence interval, 1.056-2.040).

Allele frequency was compared between the good recovery group and poor recovery groups among patients with SD. No significant difference was found between the good and poor recovery groups (Table 5).

DISCUSSION

We conducted a case-control retrospective study in which the OR analysis was mainly used to predict disease susceptibility. This study demonstrated that the UCP2 polymorphism (rs 660339) has a significant association with SD risk. UCPs are the members of the anion carrier protein family and are located in the inner mitochondrial membrane. UCP inhibits insulin secretion and may be related to obesity, β cell damage, and diabetes. UCP exerts a protective effect against free radicals, and it is related to thermogenesis [23]. Previous studies have investigated the association between UCP2 gene polymorphisms and the presence of diabetic complications. The polymorphisms of the UCP2 gene have been reported to be significantly associated with a risk of proliferative diabetic retinopathy in Brazilian patients with type 1 and type 2 diabetes mellitus [24]. The mRNA expression of UCP3, UCP4, and especially UCP2 is upregulated after a unilateral labyrinthectomy in the inner ear of rats. Furthermore, the expression of UCP2, 3, and 4 is upregulated after a systemic administration of kanamycin in the mouse inner ear [17, 18]. Sugiura et al. [19] have described that the UCP2 T allele (rs660339) was also associated with presbycusis as in SD. The neuroprotective part against oxidative stress may be associated with the hearing function. Conversely, it is assumed that the UCP2 C allele may have a protective role to prevent sensorineural hearing loss by strengthening the antioxidative function. This was suggested in the present study of SD as well as another study of presbycusis. Manche et al. [25] have also reported that UCP2 (G-866 A)—another common polymorphism of this gene—was associated with presbycusis.

CONCLUSION

The present study demonstrates that UCP2 polymorphisms may be associated with the risk of developing SD, as suggested by the results of our multivariate analysis with moderating variables. Future studies including more cases are warranted.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committees of Nagoya University (370-4) and by the National Center for Geriatrics and Gerontology (nos.14, 52, and 74).

Informed Consent: Written informed consent was obtained from all subjects who participated in this study.

Peer-review: Externally peer-reviewed.


Acknowledgements: We would like to give our sincere thanks to the researchers from Section of National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA), National Center for Geriatrics and Gerontology who were involved in data collection and analyses.

Conflict of Interest: The authors have no conflict of interest to declare.
REFERENCES


INTRODUCTION

Increase in the elderly population is a global issue. Many diseases may be associated with aging of an individual. Age-related hearing impairment (ARHI) is a major disability among older individuals. The prevalence of hearing impairment has been reported to be 34% in individuals 65 years of age or older, and it increases to 72% in individuals 85 years of age or older [1, 2]. In Taiwan, the prevalence of hearing impairment is reportedly as high as 78% among individuals 65 years of age or older [3]. The high prevalence of ARHI in Taiwan attracted our attention, and we initiated the present study.

ARHI is a multifactorial condition, representing the end result of multiple intrinsic (e.g., genetic predisposition) and extrinsic (e.g., noise exposure) factors acting on the inner ear leading to the accumulation of damages in the pathway of auditory signal transduction [4]. Chronic inflammation, hypoxia, noise, and toxic substances may increase oxidative stress in the inner ear, cause the production of reactive oxygen species, lead to necrosis and apoptosis of inner ear cells, and result in ARHI [4-6]. In the auditory pathway, glutamate is the primary neurotransmitter; however, overstimulation by glutamate may be toxic. Excess glutamate in the extracellular space below the inner hair cells (IHC) could result in permeability changes in the postsynaptic membrane of den-
drites and cause osmotic imbalance, edema, and auditory dendrite destruction [7, 8]. The administration of glutamate antagonists may protect postsynaptic targets and reduce functional impairment [5], indicating that the interaction of glutamate with its receptors may be involved in the mechanism of hearing impairment. Metabotropic glutamate receptors (mGluRs) are receptors located in the neurons receiving glutamatic signals from IHC and coupled to effector systems through GTP-binding proteins, which can modulate or fine-tune the activity at the synapse [9].

To date, eight subtypes of mGluRs have been identified. Some studies have reported that the single nucleotide polymorphism (SNP) of metabotropic glutamate receptor 7 (GRM7) gene is related to ARHI [10-13]. In the above studies, the SNP rs11928865 was reported to be significantly associated with susceptibility to ARHI, regardless of whether the studies were conducted in Caucasians [10, 12, 13] or Asians [11]. However, the minor allele frequency (MAF) of rs11928865 was as high as 0.32 in Caucasians, whereas it was as low as 0.14–0.20 in Asians [14]. With the high prevalence of ARHI (78%) in Taiwan and the low MAF (0.14–0.20) of rs11928865 in Asians, we hypothesized that there are some GRM7 SNPs, which have a higher MAF and are closely linked to rs11928865, associated with susceptibility to ARHI.

The goals of the present study were 1) to generate data of the genetic distribution of target SNPs in the Taiwanese population and 2) to determine common GRM7 SNPs that are associated with susceptibility to ARHI in our population. We hope that the findings of the present study can be applied in the development of screening and treatment options of ARHI in the future.

MATERIALS and METHODS

Participants

The participants of this study were clients who had undergone national annual health examinations performed by the health management center in a metropolitan hospital, from March 17 to May 19, 2015. The national annual health examinations were free of charge for participants older than 65 years of age, with funding provided by the Health Promotion Administration, Ministry of Health and Welfare, Taiwan. We recruited volunteers who were 65 years of age or older and who agreed to participate in the study and undergo additional pure tone audiometric tests and take self-reported questionnaires about the history of noise exposure and medical history of ear diseases. Participants with a history of noise exposure and/or otologic diseases were excluded from this study later during data analysis. Participants with dementia and those who could not sit independently in order to undergo the audiometric tests were also excluded from this study.

Audiometric Assessments

Pure tone audiometry was performed in sound-attenuating booths by trained technicians using standard procedures that met the requirements of the Council of Labor Affairs, Executive Yuan, Taiwan. The audiometric data were recorded at frequencies of 500, 1000, 2000, 3000, 4000, 6000, and 8000 Hz. Bone conduction (BC) audiograms were also recorded at frequencies of 500, 1000, 2000, 3000, and 4000 Hz for those whose air conduction (AC) hearing levels were >25 decibel hearing level (dBHL). The highest BC frequency achieved was 4000 Hz because of technical and physical limitations. The BC hearing levels were used for evaluating the severity of hearing loss in the older adults. For those with AC hearing levels ≤25 dBHL, the AC hearing levels were adopted. For those with BC hearing levels beyond the limit of audiometric testing capacity, the upper limit of the BC testing capacity was adopted (i.e., 65 dBHL). The BC hearing level was adopted for hearing assessments to minimize the possibility of conductive hearing loss. The speech frequency (500, 1000, 2000, and 4000 Hz) pure tone average (PTA) was calculated for each side. The PTA of the better hearing ear was used to evaluate the severity of the hearing impairment, as per the World Health Organization grading system (http://www.who.int/pbd/deafness/hearing_impairment_grades/en/). According to our earlier study, individuals may be perceptive to hearing impairment at a hearing level of >35 dBHL [11]. The participants were categorized into three groups: normal control (PTA≤25 dBHL), indolent hearing loss (PTA>25 dBHL but ≤35 dBHL), and perceptive hearing loss (PTA>35 dBHL). To identify the phenotypes more clearly, we selected the perceptive hearing loss group (ARHI group) as the case group and the normal control group as the control later for case–control analyses.

Methods

SNP selection

The target SNPs were searched using the software GLIDERS [15], with the following criteria: Asians (JPT+CHB), MAF≥0.25, linkage disequilibrium value (LD value; r²) with rs11928865 ≥0.5, and within 20 kb to rs11928865. Three SNPs were found with the above criteria (Table 1). Four SNPs (rs1353828, rs9814809, rs9880404, and rs11928865) were adopted for genetic analysis in the present study.

Genotyping

Blood samples were obtained from all the participants with written consent. Each specimen was collected in an ethylenediaminetetraacetic acid tube and was centrifuged (2000 g, 20 min). The buffy coat was isolated, and DNA was extracted using a commercial DNA extraction kit (Gentra Corp., Minneapolis, Minn, USA). Genotypes for the selected polymorphisms were screened using the ABI TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA). The targets were sequenced using ABI TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA).

Table 1. Target SNPs found by the searching engine GLIDERS

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome</th>
<th>Position (bp)</th>
<th>MAF</th>
<th>Distance (from rs11928865)</th>
<th>r²</th>
<th>D’</th>
<th>χ²</th>
<th>p</th>
<th>P(BFC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1353828</td>
<td>3</td>
<td>7,144,453</td>
<td>0.271</td>
<td>13kb</td>
<td>0.52</td>
<td>0.95</td>
<td>0.572</td>
<td>5.35e-30</td>
<td>3.17e-18</td>
</tr>
<tr>
<td>rs9814809</td>
<td>3</td>
<td>7,145,741</td>
<td>0.271</td>
<td>15kb</td>
<td>0.52</td>
<td>0.95</td>
<td>0.572</td>
<td>5.35e-30</td>
<td>3.17e-18</td>
</tr>
<tr>
<td>rs9880404</td>
<td>3</td>
<td>7,150,878</td>
<td>0.268</td>
<td>20kb</td>
<td>0.51</td>
<td>0.95</td>
<td>0.570</td>
<td>2.23e-29</td>
<td>1.32e-17</td>
</tr>
</tbody>
</table>

BFC: Bonferroni corrected; MAF: minor allele frequency; SNPs: single nucleotide polymorphisms
Calif., USA) The extracted DNA and genotyping assays were added to TaqMan universal PCR master mix (Roche, Branchburg, N.J., USA) according to the manufacturer’s instructions. The genotyping procedures were then performed using ABI PRISM–7500 realtime PCR system (Applied Biosystems). The results were analyzed using ABI 7500 System sequence detection software, version 1.2.3 (Applied Biosystems).

Statistical Analysis
All data were analyzed using the Statistical Packages for the Social Sciences software package, version 20.0 (IBM Corp.; Armonk, NY, USA). Continuous data were analyzed using independent sample Student’s t-tests. Categorical data were computed using two-sided χ2 tests. Genetic analyses were performed using the PLINK software [16]. Multivariate logistic regression analyses were used to explore the odds ratios of the genotypes on ARHI. The level of statistical significance was set at p<0.05.

Ethical Considerations
All participants provided written informed consent. No private personal information is identifiable in the data. This study was approved by the institutional review board of our institute (IRB approval No.: KMUHIRB-GV103069).

RESULTS
A total of 602 participants, including 325 (54.0%) males and 277 (46.0%) females, were recruited in the present study. The average age of the participants was 72.08±5.75 years. The average hearing threshold of the better ear was 29.20±12.03 dBHL. The demographic data, including the groups of hearing impairment, history of hearing hazard, and alleles and genotypes of the participants, are presented in Table 2.

Case-Control Analysis
After screening the histories of noise exposure and otologic diseases in the participants, 187 were excluded. Of the remaining participants, 106 were classified into the ARHI (case) group, whereas 190 participants with normal hearing levels were classified into the control group.

Allele Analysis
The distributions of the alleles of the target SNPs are presented in Table 3. For all four target SNPs, the proportion of the minor alleles seemed to be slightly higher in the case group. The distributions of the alleles of the target SNPs followed the Hardy–Weinberg equilibrium. However, no significant difference was found in the distributions of alleles between the case and control groups (p>0.05).

Genotype Analysis
The distributions of genotypes of the SNP rs1353828, rs9814809, and rs9880404 were significantly different between the case and control groups. However, there was no significant difference for rs11928865 between the groups. The patterns of the effects for the SNPs on ARHI were analyzed, and the odds ratios for the protective effects were also computed. The minor alleles of the SNP rs1353828, rs9814809, and rs9880404 were associated with hearing loss in a dominant pattern. However, after adjustment for age and sex, only rs9880404 showed statistical significance in association with susceptibility to

### Table 2. Demographics of the participants

<table>
<thead>
<tr>
<th>Number (%)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>72.30±5.84</td>
<td>71.83±5.66</td>
<td>72.08±5.75</td>
</tr>
<tr>
<td>PTA of better ear (dBHL)</td>
<td>32.15±12.47</td>
<td>27.46±11.75</td>
<td>29.20±12.03</td>
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<tr>
<td>Grouping</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Perceptive HL (&gt;35 dB)</td>
<td>111</td>
<td>59</td>
<td>170</td>
</tr>
<tr>
<td>Indolent HL (≤35 dB, &gt;25 dB)</td>
<td>92</td>
<td>72</td>
<td>164</td>
</tr>
<tr>
<td>Control (≤25 dB)</td>
<td>122</td>
<td>146</td>
<td>268</td>
</tr>
<tr>
<td>Hazard history†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noise exposure</td>
<td>109 (74.1)</td>
<td>38 (25.9)</td>
<td>147</td>
</tr>
<tr>
<td>Ear disease</td>
<td>53 (57.6)</td>
<td>39 (42.4)</td>
<td>92</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>rs11928865</th>
<th>rs1353828</th>
<th>rs9814809</th>
<th>rs9880404</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>214 (65.8)</td>
<td>189 (68.2)</td>
<td>403 (66.9)</td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>102 (31.4)</td>
<td>80 (28.9)</td>
<td>182 (30.2)</td>
<td></td>
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<tr>
<td>AA</td>
<td>8 (2.5)</td>
<td>6 (2.2)</td>
<td>14 (2.3)</td>
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<td>1 (0.3)</td>
<td>2 (0.7)</td>
<td>3 (0.5)</td>
<td></td>
</tr>
<tr>
<td>rs11928865</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1353828</td>
<td>AA</td>
<td>170 (52.3)</td>
<td>162 (58.5)</td>
<td>332 (55.1)</td>
</tr>
<tr>
<td>GC</td>
<td>133 (40.9)</td>
<td>96 (34.7)</td>
<td>229 (38.0)</td>
<td></td>
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<tr>
<td>CC</td>
<td>19 (5.8)</td>
<td>16 (5.8)</td>
<td>25 (5.8)</td>
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<tr>
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<td>3 (1.1)</td>
<td>6 (1.0)</td>
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<tr>
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<td>CC</td>
<td>173 (53.2)</td>
<td>163 (58.8)</td>
<td>336 (55.8)</td>
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<tr>
<td>CT</td>
<td>130 (40.0)</td>
<td>96 (34.7)</td>
<td>226 (37.5)</td>
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<td>19 (5.8)</td>
<td>16 (5.8)</td>
<td>35 (5.8)</td>
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<td>Alleles</td>
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<td>rs9814809</td>
<td>rs9880404</td>
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<tr>
<td>T</td>
<td>530 (81.8)</td>
<td>458 (83.3)</td>
<td>988 (82.5)</td>
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<tr>
<td>A</td>
<td>118 (18.2)</td>
<td>92 (16.7)</td>
<td>210 (17.5)</td>
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<tr>
<td>rs1353828</td>
<td>A</td>
<td>473 (73.4)</td>
<td>420 (76.6)</td>
<td>893 (74.9)</td>
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<tr>
<td>C</td>
<td>171 (26.6)</td>
<td>128 (23.4)</td>
<td>299 (25.1)</td>
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</tr>
<tr>
<td>rs9814809</td>
<td>G</td>
<td>473 (73.4)</td>
<td>420 (76.6)</td>
<td>893 (74.9)</td>
</tr>
<tr>
<td>C</td>
<td>171 (26.6)</td>
<td>128 (23.4)</td>
<td>299 (25.1)</td>
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</tr>
<tr>
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<td>C</td>
<td>476 (73.9)</td>
<td>422 (76.7)</td>
<td>898 (75.2)</td>
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<tr>
<td>T</td>
<td>168 (26.1)</td>
<td>128 (23.3)</td>
<td>296 (24.8)</td>
<td></td>
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</tbody>
</table>

†Some participants may have both noise exposure and ear disease histories
ARHI. The adjusted odds ratio of rs9880404 TT and TC genotypes to CC genotype (wild type) was 1.832 (p=0.048). The results of genotype analyses are presented in Tables 3 and 4.

The haplotypes were also analyzed. However, no specific haplotype was associated with susceptibility to ARHI.

DISCUSSION
In the present study, we found that GRM7 SNP rs9880404 is associated with susceptibility to ARHI. Compared with the CC genotype (wild type), participants with the TT and TC genotypes of SNP rs9880404 may have an increased risk for ARHI. The findings correspond to our hypothesis that some GRM7 SNPs closely related to rs11928865 are associated with susceptibility to age-related hearing loss. Although rs1353828 and rs9814809 were found not to be associated with susceptibility to ARHI, three SNPs (rs1353828, rs9814809, and rs9880404) were found to be closely related to each other. The SNPs rs1353828 and rs9814809 were in LD with r^2=1.0, where the A and C alleles in rs1353828 always correspond to the G and C alleles in rs9814809, respectively. The SNP rs9880404 was also in LD with rs1353828 and rs9814809 with r^2=0.98. The relationships among these SNPs illustrated by Haploview are presented in Figure 1.

In the present study, however, SNP rs11928865 was found not to be associated with ARHI. This finding was different from that in other previously published studies, which confirmed that rs11928865 is associated with susceptibility to ARHI [10-13]. Ethnical differences might be the primary cause for these differing results. Reportedly, the global MAF of rs11928865 is 0.256 [14], whereas the MAFs of this SNP are 0.292 in Europeans and 0.175 in the population of the present study. In Asians, the MAF of this SNP was found to be smaller than that in Europeans. The difference in MAFs among races may interfere with the expression of clinical symptoms. In the study by Luo et al., the authors concluded that rs11928865 is associated with susceptibility to ARHI of high-tone loss audiometric patterns in a Chinese population [11]. In their study, however, rs11928865 was found not to be

<table>
<thead>
<tr>
<th>Table 3. Analyses of alleles and genotypes between ARHI and control groups</th>
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<tr>
<td></td>
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<tr>
<td>Number</td>
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<tr>
<td>Age (Years)</td>
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<tr>
<td>PTA of better ear (dBHL)</td>
</tr>
<tr>
<td>SNPs</td>
</tr>
<tr>
<td>rs11928865</td>
</tr>
<tr>
<td>Alleles</td>
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<td></td>
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<tr>
<td>Genotypes</td>
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</tr>
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<tr>
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<td>Genotypes</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
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</tbody>
</table>

†: Chi-square
‡: Logistic regression; adjusted for sex and age; wild type as reference

The haplotypes were also analyzed. However, no specific haplotype was associated with susceptibility to ARHI.
associated with ARHI of flat loss audiometric patterns. Due to technical and physiological limitations, the highest BC frequency in the present study was limited to 4000 Hz. The phenotype of ARHI was defined by the speech frequency (500, 1000, 2000, and 4000 Hz) PTA of the better hearing ear, which was more similar to the flat loss audiometric pattern. Hence, the insignificant association of rs11928865 with ARHI in the present study did not conflict with the findings of earlier published studies.

The present study supported the findings of GRM7 association with ARHI [10-13]. Metabotropic glutamate receptor 7 (mGluR7) is a member of the mGluR III group. It is expressed at the highest levels in the adult brain during hippocampal formation, in the cerebral cortex, and in the cerebellum [17]. The mechanism by which mGluR7 affects hearing is still unclear. High concentrations of glutamate may cause overstimulation and excitotoxicity in the auditory system, leading to several forms of progressive hearing loss, such as noise-induced hearing loss and ARHI [8]. High concentrations of glutamate may activate the expression of mGluRs. Animal studies have found that the activation of mGluRs regulates intracellular calcium concentration in cochlear nucleus neurons [18-20]. The physical level of the calcium concentration of cochlear nucleus neurons is maintained by the mGluR-mediated activation of protein kinase A and C, whereas the deprivation or interruption of the calcium concentration-regulating mechanism are predictive of subsequent cell death [20]. The SNPs in the present study, including rs11928865, were intron variants located on chromosome 3. Perhaps the variations of these SNPs modified the expression of GRM7 in some way, or these SNPs were linked to the disequilibrium of the variants in other GRM genes. Further studies on the expressions or functional analyses of mGluRs might help answer these questions.

The major limitation of the present study is the small sample size. There were only one or two participants with the genotype of homogenous minor alleles for SNPs in the case group. Hence, a small difference in the case number may affect the statistical results enormously. Enlarging the sample size may help enhance the validity of the results in the present study. The recruitment of participants from multiple institutes in future studies should be considered.

CONCLUSION
The genetic polymorphisms of GRM7 are associated with susceptibility to ARHI. The SNP rs9880404 was found to be associated with increased risk for ARHI in a dominant pattern. SNPs rs11928865, rs1353828, and rs9814809 were found not to be associated with susceptibility to ARHI in the present study. Unlike the results of the present study, SNP rs11928865 was reported to be associated with susceptibility to ARHI in European studies. The significance of SNPs in relation to susceptibility to ARHI in the present study (Asian) differed from that in European studies. Further genome-wide association studies with a larger population in our nation may help find highly ranked SNPs associated with ARHI specifically for Taiwanese or Asians.

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Review Board of Kaohsiung Medical University Hospital.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was supported by Teaching and Research Center of Kaohsiung Municipal Hsiao-Kang Hospital (Grant number: KMHK-103-009).

Table 4. Trend analysis of hereditary patterns

<table>
<thead>
<tr>
<th></th>
<th>ARHI</th>
<th>Control</th>
<th>p*</th>
<th>OR (95% CI)</th>
<th>p* (Adjusted)</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11928865</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant (AA+AT):TT</td>
<td>34:71</td>
<td>51:137</td>
<td>0.343</td>
<td>0.777 (0.462–1.308)</td>
<td>0.181</td>
<td>0.643 (0.337–1.227)</td>
</tr>
<tr>
<td>Recessive AA: (AT+TT)</td>
<td>1:104</td>
<td>5:183</td>
<td>0.343</td>
<td>0.352 (0.041–3.053)</td>
<td>0.181</td>
<td>1.555 (0.815–2.966)</td>
</tr>
<tr>
<td>rs1353828</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant (CC+AC):AA</td>
<td>52:53</td>
<td>67:120</td>
<td>0.023*</td>
<td>1.757 (1.081–2.855)</td>
<td>0.066</td>
<td>1.745 (0.964–3.157)</td>
</tr>
<tr>
<td>Recessive CC:(AC+AA)</td>
<td>2:103</td>
<td>12:175</td>
<td>0.103</td>
<td>0.283 (0.062–1.290)</td>
<td>0.166</td>
<td>0.305 (0.057–1.637)</td>
</tr>
<tr>
<td>rs9814809</td>
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<tr>
<td>Dominant (CC+CG):GG</td>
<td>52:53</td>
<td>67:120</td>
<td>0.023*</td>
<td>1.757 (1.081–2.855)</td>
<td>0.066</td>
<td>1.745 (0.964–3.157)</td>
</tr>
<tr>
<td>Recessive CC:(CG+GG)</td>
<td>2:103</td>
<td>12:175</td>
<td>0.103</td>
<td>0.283 (0.062–1.290)</td>
<td>0.166</td>
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<td>rs9880404</td>
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<tr>
<td>Dominant (TT+CT):CC</td>
<td>51:54</td>
<td>67:120</td>
<td>0.034*</td>
<td>1.692 (1.041–2.749)</td>
<td>0.048*</td>
<td>1.832 (1.005–3.339)</td>
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<tr>
<td>Recessive TT:(CT+CC)</td>
<td>2:103</td>
<td>12:175</td>
<td>0.103</td>
<td>0.283 (0.062–1.290)</td>
<td>0.157</td>
<td>0.295 (0.055–1.598)</td>
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</tbody>
</table>

*: Logistic regression
‡: Adjusted for age and sex
*: p<0.05
REFERENCES


Pentoxifylline versus Steroid Therapy for Idiopathic Sudden Sensorineural Hearing Loss with Diabetes

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China Medical University Hospital, Otolaryngology Head and Neck Surgery, Taichung City, Taiwan

OBJECTIVES: To compare the efficacy of pentoxifylline with that of conventional steroid therapy in diabetic patients with idiopathic sudden sensorineural hearing loss (ISSNHL) and to compare blood sugar levels during hospitalization.

MATERIALS and METHODS: Medical charts were retrospectively reviewed for all diabetic patients admitted to one institution for ISSNHL between 2000 and 2015. We analyzed 298 cases; 50 patients received pulse steroid treatment (steroid group) and 248 received intravenous administration of pentoxifylline only (pentoxifylline group). Hearing change was evaluated by comparing the initial hearing tests with follow-up hearing tests for up to 3 months. Blood sugar levels were also compared between the 2 groups.

RESULTS: At 3 months post-treatment, the degree of hearing recovery was similar between the 2 groups. The pure-tone average was improved from baseline by 17.9±21.2 dB in the steroid group and 18.9±20.7 dB in the pentoxifylline group (p=0.776); hearing recovery rates were also similar (40% vs 39.1%; p=0.826). During hospitalization, average fasting blood sugar levels were higher (203.9±92.0 vs 174.4±54.8 mg/dL; p=0.033) and acute hyperglycemia was more common (48.0% vs 33.1%; p=0.044) with steroid versus pentoxifylline treatment.

CONCLUSION: Hearing recovery rates did not significantly differ between steroid and pentoxifylline treatment in diabetic patients with ISSNHL, but pentoxifylline appeared to be associated with better blood sugar control.

KEYWORDS: Idiopathic sudden sensorineural hearing loss, diabetes mellitus, steroid, pentoxifylline, blood glucose
Steroids are known to intensify hyperglycemia in patients with a history of DM and also lead to DM in those without any history of hyperglycemia [8]. In some cases, steroids trigger acute complications such as diabetic ketoacidosis and hyperosmolar hyperglycemic state [9].

Cochlear ischemia has been considered to be a potential etiology of ISSNHL. Therefore, vasoactive agents, such as Ginkgo biloba extract, dextran, and pentoxifylline, have been used with an aim to gain more blood flow in the cochlea [10, 11]. Pentoxifylline has been reported to have an ability of increasing erythrocyte flexibility, reducing blood viscosity, and increasing microcirculatory flow [12, 13].

DM can cause microvascular injuries and other microcirculatory disorders including unexpected increment of blood viscosity and thrombotic and embolic events [14]. Notably, the prevalence of DM in adults has increased from 4.7% in 1980 to 8.5% in 2014 [15]. This increase in DM prevalence warrants careful assessment of treatment outcomes and close monitoring of any treatment-related complications of ISSNHL in diabetic patients.

The aim of this retrospective study was to evaluate the efficacy of pentoxifylline with that of conventional corticosteroid therapy in diabetic patients with ISSNHL and to monitor their blood sugar control during hospitalization.

**MATERIALS and METHODS**

Medical charts were retrospectively reviewed for all diabetic patients admitted for ISSNHL to a single tertiary hospital between 2000 and 2015. A total of 298 patients were included in this study according to the inclusion criteria presented in Table 1.

Patients with an identified etiology, recurrent hearing loss, bilateral hearing loss, newly diagnosed or uncontrolled diabetes, concomitant middle ear disease, or previous surgery in the affected ear were excluded. Any patients receiving concomitant systemic steroid and pentoxifylline therapy were also excluded. This study was approved by the Institutional Review Board of the China Medical University Hospital. Informed consent is not necessary because of the retrospective nature of this study.

Detailed profiles were constructed for each patient that included demographic data, affected ear, duration from the onset of hearing loss to the beginning of therapy, treatment modalities, comorbidities, regular blood tests, and any associated symptoms (Table 2). Details

### Table 1. Inclusion criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Pentoxifylline group (n=248)</th>
<th>Corticosteroid group (n=50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥18 years old</td>
<td></td>
<td></td>
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<tr>
<td>Acute onset of ≥30 dB unilateral sensorineural hearing loss, over at least 3 contiguous frequencies, occurring within 72 hours</td>
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<tr>
<td>No identifiable cause for the hearing impairment†</td>
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<tr>
<td>Not recurrent sudden hearing loss</td>
<td></td>
<td></td>
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<tr>
<td>No other neurological signs (except for dizziness, vertigo, and tinnitus)</td>
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<tr>
<td>Availability of pre-treatment and post-treatment audiograms up to 3 months</td>
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<tr>
<td>Have a history of DM with medication control (either OHA or Insulin therapy)</td>
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</table>

†Meniere’s disease, cochlear trauma, autoimmune disease, syphilis, Lyme disease, ototoxic drug, and perilymphatic fistula

DM: diabetes mellitus; OHA: oral hypoglycemic agent

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pentoxifylline group</th>
<th>Corticosteroid group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>61.4±10.8</td>
<td>59.2±11.1</td>
<td>0.192</td>
</tr>
<tr>
<td>Sex: males: females, n (%)</td>
<td>125:123 (50.4:49.6)</td>
<td>25:27 (46:54)</td>
<td>0.570</td>
</tr>
<tr>
<td>Affected ear: left:right, n (%)</td>
<td>121:127 (48.8:51.2)</td>
<td>31:19 (62:38)</td>
<td>0.088</td>
</tr>
<tr>
<td>Onset to treatment (days)</td>
<td>6.0±7.4</td>
<td>6.6±5.6</td>
<td>0.572</td>
</tr>
<tr>
<td>Dizziness or Vertigo, n (%)</td>
<td>126 (50.8)</td>
<td>28 (56.0)</td>
<td>0.503</td>
</tr>
<tr>
<td>Pure-tone threshold at each frequency (dB)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 kHz</td>
<td>68.9±24.9</td>
<td>76.3±23.6</td>
<td>0.057</td>
</tr>
<tr>
<td>0.5 kHz</td>
<td>77.5±22.3</td>
<td>81.2±19.0</td>
<td>0.275</td>
</tr>
<tr>
<td>1 kHz</td>
<td>81.1±22.2</td>
<td>85.3±18.7</td>
<td>0.221</td>
</tr>
<tr>
<td>2 kHz</td>
<td>78.9±23.0</td>
<td>82.7±21.2</td>
<td>0.291</td>
</tr>
<tr>
<td>4 kHz</td>
<td>83.7±24.1</td>
<td>85.5±22.1</td>
<td>0.644</td>
</tr>
<tr>
<td>8 kHz</td>
<td>88.4±20.9</td>
<td>90.4±17.6</td>
<td>0.501</td>
</tr>
<tr>
<td>PTA (dB) of affected ear</td>
<td>80.3±20.7</td>
<td>83.8±18.9</td>
<td>0.297</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.6±2.2</td>
<td>8.7±1.2</td>
<td>0.939</td>
</tr>
<tr>
<td>FBS on Day 1 (mg/dL)</td>
<td>220.1±112.6</td>
<td>250.0±98.0</td>
<td>0.082</td>
</tr>
<tr>
<td>DM therapy: OHA:insulin, n (%)</td>
<td>191:57 (77:23)</td>
<td>40:10 (80:20)</td>
<td>0.714</td>
</tr>
</tbody>
</table>

DM: diabetes mellitus; PTA: pure-tone threshold average, determined by calculating the mean of the 0.5, 1, 2, and 4 kHz thresholds. HbA1c: glycated hemoglobin; FBS: fasting blood sugar; OHA: oral hypoglycemic agent
such as fasting blood sugar (FBS) levels, types of DM medications used, HbA1c values within the previous 3 months, and any acute complications such as hyperosmolar hyperglycemic state and ketoacidosis were also recorded. Clinical examinations were performed in all patients, and those with identifiable etiologies of hearing loss were excluded.

Patients in the steroid group received IV hydrocortisone (China Chemical & Pharmaceutical Company, Taipei, Taiwan) 300 mg daily on Days 1–3, followed by oral prednisolone 60 mg on Day 4 and 50 mg on Day 5. After discharge on Day 5, the patients commenced oral prednisolone on Day 6 at a dose of 40 mg, which was decreased thereafter by 10 mg daily until a daily maintenance dose of 10 mg continuing up to Day 14. The pentoxifylline group received IV pentoxifylline (Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany) 300 mg daily for 5 days, followed by oral pentoxifylline 1200 mg daily for 9 days.

Pure-tone thresholds for air conduction were conducted at 0.25, 0.5, 1, 2, 4, and 8 kHz frequencies. The pure-tone average (PTA) was determined by calculating the mean of the 0.5, 1, 2, and 4 kHz thresholds. Audiometric data were recorded at the time of admission before treatment and at 1, 4, and 12 weeks after treatment initiation.

Hearing change during treatment was assessed by comparing the hearing test results before the treatment with those at 3 months. Pure-tone threshold improvements in each individual tone (0.25, 0.5, 1, 2, 4, and 8 kHz) were recorded. Patients were also categorized into complete, partial, or no recovery of hearing groups according to the definition proposed by the American Academy of Otolaryngology-Head and Neck Surgery Foundation (AAO-HNSF) Clinical Practice Guidelines: 1) complete recovery, post-treatment PTA return to within 10 dB hearing level of the normal ear; 2) partial recovery, defined in 2 ways: 1) for ears that were nonserviceable (≥50 dB on PTA and ≤50% speech discrimination score) after the onset of hearing loss, return to serviceable hearing will be considered as partial recovery; 2) for ears that were serviceable, a 10 dB improvement in PTA will be considered as partial recovery; and 3) no recovery, post-treatment PTA improvement was ≤10 dB. Hearing level of the normal ear recorded before treatment initiation was used as baseline for calculating hearing recovery.

### Results

At 3 months’ follow-up, the average hearing gain was 22.7±24.9, 25.2±24.3, 21.5±21.3, 19.0±19.6, 17.8±20.9, and 7.6±13.5 dB, respectively, for audiogram frequencies of 0.25, 0.5, 1, 2, 4, and 8 kHz in the steroid group. The corresponding hearing gain in the pentoxifylline group was 20.8±27.5, 20.6±39.3, 21.0±22.9, 17.6±20.6, 13.8±20.4, and 9.5±16.1 dB, respectively. Average PTA improvements at 12 weeks in the steroid and pentoxifylline groups were 17.9±21.2 and 18.9±20.7 dB, respectively; the between-group difference was not significant (p=0.776). Treatment results at 1, 4, and 12 weeks are shown in Table 3. Hearing improvement at a specific frequency did not significantly differ between the groups.

### Table 3. Hearing gain after treatment for 1 week, 4 weeks, and 12 weeks

<table>
<thead>
<tr>
<th>Hearing gain (dB)</th>
<th>Pentoxifylline group (n=248)</th>
<th>Corticosteroid group (n=50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 week</td>
<td>4 weeks</td>
</tr>
<tr>
<td>0.25 kHz</td>
<td>68.9±24.9</td>
<td>8.0±21.4</td>
<td>15.1±26.1</td>
</tr>
<tr>
<td>0.5 kHz</td>
<td>77.5±22.3</td>
<td>8.9±20.4</td>
<td>17.2±24.8</td>
</tr>
<tr>
<td>1 kHz</td>
<td>81.1±22.2</td>
<td>8.4±16.7</td>
<td>16.0±20.7</td>
</tr>
<tr>
<td>2 kHz</td>
<td>78.9±23.0</td>
<td>8.1±15.9</td>
<td>14.1±19.5</td>
</tr>
<tr>
<td>4 kHz</td>
<td>83.7±24.1</td>
<td>5.1±16.9</td>
<td>10.4±19.6</td>
</tr>
<tr>
<td>8 kHz</td>
<td>88.4±20.9</td>
<td>2.6±12.8</td>
<td>6.8±15.9</td>
</tr>
<tr>
<td>PTA</td>
<td>80.8±20.7</td>
<td>7.6±15.2</td>
<td>14.4±18.8</td>
</tr>
</tbody>
</table>

PTA: pure-tone threshold average, determined by calculating the mean of the 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz thresholds. NS: not significant between 2 groups (at 1 week, 4 weeks, and 12 weeks).

*The steroid group had significant hearing gain at 4 kHz 1 week after treatment (p=0.011), but had no significant hearing gain at 4 and 12 weeks after treatment.
with ISSNHL and DM [7]. They received oral prednisolone for 10 days for diabetic ISSNHL patients. It steroid treatment proved to be as suitable alternative to systemic steroids as an initial treatment.

Acute hyperglycemia‡, n (%) 82 (33.1) 24 (48.0) 0.044

Table 5 demonstrates average FBS levels and occurrences of acute hyperglycemia between the treatment groups. The mean FBS levels were 203.9±92.0 mg/dL in the steroid group and 174.4±54.8 mg/dL in the pentoxifylline group (p=0.033). Almost half (48%) of all steroid-treated patients developed acute hyperglycemia, whereas this occurred in significantly fewer pentoxifylline-treated patients (33.1%; p=0.044). The average FBS values during hospitalization were higher in the steroid group despite strict adherence to insulin therapy. Acute hyperglycemia episodes were also more common in the steroid group.

Table 5. Blood sugar control

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Pentoxifylline group (n=248)</th>
<th>Corticosteroid group (n=50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete recovery, n (%)</td>
<td>65 (26.2)</td>
<td>12 (24)</td>
<td>0.826</td>
</tr>
<tr>
<td>Partial recovery, n (%)</td>
<td>32 (12.9)</td>
<td>8 (16)</td>
<td></td>
</tr>
<tr>
<td>No recovery, n (%)</td>
<td>151 (60.9)</td>
<td>30 (60)</td>
<td></td>
</tr>
</tbody>
</table>

FBS: fasting blood sugar
‡FBS >300 mg/dL or FBS >200 mg/dL in those with HbA1c <8% within the 3 months prior to hospitalization

except for the significant hearing gain in the steroid group at 4 kHz at 1 week after treatment.

AAO-HNSF values for the steroid group demonstrated a complete recovery rate of 24%, a partial recovery rate of 16%, and a no recovery rate of 60%. The corresponding values for the pentoxifylline group were 26.2%, 12.9%, and 60.9%, respectively. The overall recovery rates were 40% for the steroid group and 39.1% for the pentoxifylline group (p=0.826). Table 4 summarizes the hearing recovery of the 2 groups after 3 months. Statistical differences of the hearing recovery rates were not significant between the 2 groups.

Table 4. Hearing recovery after 3 months†

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Pentoxifylline group (n=248)</th>
<th>Corticosteroid group (n=50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete recovery, n (%)</td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>No recovery, n (%)</td>
<td>151 (60.9)</td>
<td>30 (60)</td>
<td></td>
</tr>
</tbody>
</table>

Categorized according to the AAO-HNSF definition supplied in the manuscript.

To the best of our knowledge, the efficacy of pentoxifylline treatment has not previously been assessed in diabetic patients with ISSNHL. Our retrospective comparison of pentoxifylline with corticosteroids in the treatment of diabetic patients with ISSNHL found that pentoxifylline was not inferior to corticosteroid therapy.

DM may induce thrombotic and embolic events and increase blood viscosity and is therefore a possible risk factor of SSNHL. In view of the increasing prevalence of DM worldwide, treatment of ISSNHL in diabetic patients warrants therapy that does not exacerbate blood sugar levels and add to the global DM burden.

At baseline, demographics, auditory data, and DM variables did not significantly differ between the 2 groups (Table 2). According to the results listed in Tables 3 and 4, pentoxifylline used in our treatment protocol is apparently not inferior to a 2-week corticosteroid regimen. The overall recovery rates (including partial and complete recovery) were similar for the pentoxifylline and steroid groups (39.1% vs 40%; p=0.826). In a randomized controlled trial, where the AAO-HNSF definition was used for the recovery definitions, the overall recovery rate was 55%-59% after 14 days of corticosteroid therapy. [4] Our recovery rates were lower than those observed in the above study. However, the mean age of patients in that study was lesser than that in our study group (40-42 years vs 59-61 years). Moreover, all of our patients had DM, whereas the study mentioned above did not focus on diabetic patients.

As detailed in Table 5, there were significant between-group differences in the average FBS levels and occurrences of acute hyperglycemia. The average FBS levels were higher and acute hyperglycemia was more common in the steroid group. Chronic hyperglycemia has long been considered to be related to the generation of oxidative stress and is a risk factor for accelerated atherosclerosis. However, acute blood sugar fluctuations in diabetes have recently been documented as a contributing factor in oxidative stress, which may lead to cardiovascular events. In view of the increasing prevalence of DM worldwide, treatment of ISSNHL in diabetic patients may be at a higher risk of cardiovascular events than those receiving pentoxifylline therapy. In a study, diabetic patients with ISSNHL treated with steroids needed more frequent use of insulin for blood sugar control compared with

**DISCUSSION**

Systemic steroids are the standard recommended treatment and are widely used for treating ISSNHL [4]. However, steroids have been associated with uncontrolled hyperglycemia, which limits their use in patients with DM [17]. Intratympanic injection of steroids is considered to be a suitable alternative to systemic steroids as an initial treatment for diabetic ISSNHL patients. IT steroid treatment proved to be as effective as systemic steroid therapy in a cohort of Korean patients with ISSNHL and DM [7]. They received oral prednisolone for 10 days (n=48), IV prednisolone for 7 days followed by oral prednisolone for another several days (IV group; n=32), or injections of dexamethasone into the middle ear cavity within a 2-week treatment period (IT group; n=34). No significant between-group differences were observed in hearing gain and recovery rates. At the end of treatment, the mean hearing improvements were 20 dB in the IV group, 26.2 dB in the oral treatment group, and 25.8 dB in the IT group. Recovery rates, defined as hearing recovery ≥15 dB, were 66.7% in the IV group, 72.3% in the oral treatment group, and 79.4% in the IT group. However, 1 patient in the oral group and 2 in the IV group dropped out of the study because of uncontrolled hyperglycemia. In another study, Fukui et al. [18] examined clinical and audiologic characteristics of 148 ISSNHL patients, 25 (16.2%) of whom had type 2 DM. Twelve out of 17 diabetic patients who were treated with steroids needed more frequent use of insulin therapy during the treatment period for adequate blood sugar control. A retrospective study (n=67) reported that diabetic patients with SSNHL have better improvement in low-to-middle-tone hearing loss than high-tone hearing loss [19]. Interestingly, the study results demonstrated treatment benefits with high-dose steroid therapy, and the authors concluded that the use of high-dose steroid therapy in diabetic patients with ISSNHL is recommended despite the high risk of exacerbation of blood sugar levels.

At baseline, demographics, auditory data, and DM variables did not significantly differ between the 2 groups (Table 2). According to the results listed in Tables 3 and 4, pentoxifylline used in our treatment protocol is apparently not inferior to a 2-week corticosteroid regimen. The overall recovery rates (including partial and complete recovery) were similar for the pentoxifylline and steroid groups (39.1% vs 40%; p=0.826). In a randomized controlled trial, where the AAO-HNSF definition was used for the recovery definitions, the overall recovery rate was 55%-59% after 14 days of corticosteroid therapy. [4] Our recovery rates were lower than those observed in the above study. However, the mean age of patients in that study was lesser than that in our study group (40-42 years vs 59-61 years). Moreover, all of our patients had DM, whereas the study mentioned above did not focus on diabetic patients.

As detailed in Table 5, there were significant between-group differences in the average FBS levels and occurrences of acute hyperglycemia. The average FBS levels were higher and acute hyperglycemia was more common in the steroid group. Chronic hyperglycemia has long been considered to be related to the generation of oxidative stress and is a risk factor for accelerated atherosclerosis. However, acute blood sugar fluctuations in diabetes have recently been documented as a contributing factor in oxidative stress, which may lead to cardiovascular events. In view of the increasing prevalence of DM worldwide, treatment of ISSNHL in diabetic patients may be at a higher risk of cardiovascular events than those receiving pentoxifylline therapy. In a study, diabetic patients with ISSNHL treated with steroids needed more frequent use of insulin for blood sugar control compared with
non-diabetic patients with ISSNHL. Although no acute complication such as diabetic ketoacidosis or hyperosmolar hyperglycemic state was reported in our patients during steroid therapy, close attention to blood sugar control is still necessary.

This study has some limitations. First, the nature of retrospective analysis is a potential source for selection bias. Second, it is difficult to make comparisons among studies due to lack of a standard definition of hearing recovery. In the future, randomized trials that incorporate a standard definition of recovery are needed to compare the efficacy of pentoxifylline therapy with that of conventional steroid therapy. Studies providing long-term outcomes in the treatment of ISSNHL are also warranted.

CONCLUSION
Pentoxifylline therapy in our study resulted in similar hearing improvements compared with steroid therapy in diabetic patients with ISSNHL and was also associated with superior blood sugar control during hospitalization.

REFERENCES
INTRODUCTION
The spiral ganglion (SG) are structures deputed to conduct sound-evoked neural activity from inner ear hair cells (IHCs) to the central nervous system. Approximately 95% of SGs’ auditory nerve fibers form synapses with IHCs, whereas about 5% with outer hair cells (OHCs) [1].

The SGs can be damaged by noise [2-7], drugs [8-11], electromagnetic radiation [12], oxidative stress, and aging [3, 13, 14]. The reduction of SGs may affect the quality of sound perception, and more importantly, word discrimination in humans.

Recently, Liberman and Kujawa [15, 16] have highlighted the extreme vulnerability of the afferent synapses and type I SG neurons that contact IHCs; their data suggest that the most vulnerable structures are the afferent terminals of the IHCs that connect to type I auditory nerve fibers and SGs [10, 15-17].

Nucleolus vs Nucleus Count for Identifying Spiral Ganglion in Human Temporal Bone

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University La Sapienza, Department of Oral and Maxillofacial Sciences, Rome, Italy (MR)
Toronto General Hospital, Otolaryngology Department, Toronto, Canada (RI)
“Carlo Poma” Civil Hospital, Department of Otolaryngology-Head and Neck Surgery, Mantova, Italy (LD)
Meyer Children’s Hospital, Otolaryngology unit, Florence, Italy (FT)
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OBJECTIVES: Spiral ganglion (SG) counting is used in experimental studies conducted on age-, noise-, and drug-induced sensorineural hearing loss, as well as in the assessment of cochlear implant performances. Different methods of counting have been reported, but no definite standardization of such procedure has been published. The aim of our study is to identify the best method to count human spiral ganglions (SGs).

MATERIAL and METHODS: By identification of nuclei or nucleoli as described by Schucknect, seven researchers with different experience levels counted SGs in 123 human temporal bones (TBs). Data on time of post-mortem bone removal post-mortem, methods of specimen’s fixation, decalcification, and coloration were collected to test their possible influence on human tissue. Percentage, two-tailed t-test, Spearman’s test, and one-way ANOVA were used to analyze the data.

RESULTS: Nucleoli were identified in 61% of cases, whereas nuclei were recognized in 100% of cases (p<0.005). Nucleoli presence in all four segments in the same temporal bone (TB) was observed in 69 cases (92%), whereas nuclei were identified in all four segments in 103 cases (83.7%) (p<0.001). The junior investigators requested a double check by the seniors in 25 (20.3%) cases for identifying and counting nucleoli, whereas the senior researchers showed no doubts in their identification and count. The only parameter positively affecting nucleoli identification in tissue preparation was bone removal for <12 h with respect to longer post-mortem time (p<0.001).

CONCLUSION: We suggest counting nuclei, rather than nucleoli, for spiral ganglion computation because of easier recognition of nuclei, especially in case of investigator's limited experience.

KEYWORDS: Spiral ganglion, hearing loss, count method, feasibility, accuracy
The identification and count of SGs is widely used in experimental studies conducted on age- [18-21], noise-, and drug-induced sensorineural hearing loss [22], as well as on laboratory tests on otoprotective drugs [23, 24]. The number of preserved SGs is also considered an important factor to improve performances in subjects with cochlear implant [25-27]. The correct identification and count of SGs has a relevant role in basic and clinical research.

A technique for SGs count was first described by Schuknecht [28] in 1978, and then by Merchant [29] and Nadol et al. [30] several years later. The SGs in human temporal bones (TBs) resemble fried eggs; they contain one nucleus (mean diameter: 10 µm) that has a nucleolus inside it (mean diameter: 2.5 µm) [30]. The counting of SGs is usually based on the identification of cells' nuclei, but because of the large diameter of nuclei, this method is associated with a high risk of cell double-counting. In fact, a nucleus that belongs to the same spiral ganglion (SG) may be present in two different sequential tissue sections (cut at a 10 µm distance between one and the other) because of its diameter. To avoid the bias due to double counting of structure on different planes and to section preparation, some correction coefficients have been identified [29, 31-33]. The correction coefficients consider the sections' thickness and the diameter of the structure (nucleus or nucleolus). Despite such coefficients, the exact counting of SGs remains imprecise.

Some authors proposed to count nucleoli rather than nuclei because the smaller diameter of nucleoli may reduce the risk of cell double-counting [31].

Nowadays, the choice between counting nuclei or nucleoli is personal, and is mainly related to the habit and experience of the researcher, which makes the comparison among different studies on this topic extremely unreliable.

The differences between the two methods (nuclei versus nucleoli count) have never been reported in the literature. Moreover, the effect of specimen preparation details (such as timing of bone post-mortem removal, tissue fixation, and coloring) has also never been investigated.

The aims of this study were to (a) identify the easier and more accurate method (nuclei versus nucleoli) to count SGs in human TBs, thereby comparing the results obtained by senior and junior researchers, and (b) identify one or more variables in patient character-
istics and specimen preparation that may modify the identification of nucleoli in SGs. A possible standardization of SGs counting procedure is proposed.

MATERIAL and METHODS

This study has been approved by the ethical committee of the hospital; all study procedures were conducted in accordance with the declaration of Helsinki and Institutional Review Board regulations. Informed consent was signed by donors before temporal bone (TB) removal.

Seven different researchers with different experience levels analyzed 123 TBs from 67 adults.

The senior researchers (n=3) had 3-year experience in this field, whereas the juniors (n=4) had 1-year experience. All researchers analyzed the same slides.

Bone decalcification was performed using trichloroacetic acid (TCA) or ethylenediaminetetraacetic acid (EDTA), whereas specimen fixation was done using formalin (10%).

A Zeiss light microscope with 20× and 40× magnifications was used. An ocular grid was applied on the microscope for increasing the accuracy during the count of SGs.

The presence of nucleoli inside the nuclei was counted in the SGs along the cochlear Rosenthal canal (RC), as previously described by Merchant [29] and Nadol et al [30]. The RC was divided into four segments as follows: (a) segment I, from uncus to the half of cochlea basal turn; (b) segment II, from the end of segment I until the beginning of cochlear middle turn; (c) segment III, corresponding to the middle turn of the cochlea; and (d) segment IV, corresponding to the cochlear apex (Figure 1). The identification of nuclei and nucleoli was based on their size and position inside the SG: nuclei are bigger than nucleoli and are less colored; nucleoli are found inside nuclei and have a more intense coloration (Figure 2). The prevalent structure (nucleus or nucleolus) in the four segments of RC was recorded; to define a structure as prevalent, it was necessary that it is detectable in >55% of observed SGs.

Nucleolus identification was recorded with a score: 1 if present and 0 if not.

All the TBs studied were stained by hematoxylin-eosin (HE) method.

The seven researchers were aware about the two different count methods, and they analyzed the same sections using a double-blind approach: each investigator counted SGs separately from the other, and nobody knew the number of the specimen and the patient name. For each slice, SGs count was performed by using one or the other method (nuclei and nucleoli) based on the structure that was more identifiable at a 20× magnification. The structures were assessed in each RC segment. To define their presence in the examined cochlea, it was necessary to identify the structure (nucleolus or nucleus) in at least three of the four segments of the RC.

The results of the analysis of each researcher were grouped as per researchers’ experience into senior and junior; then the overall results for different levels of experience were compared to evaluate the difference in the ability to identify nuclei or nucleoli.

Figure 2. Violet tissue coloration (hematoxylin-eosin staining). Magnification 20×. The arrow shows the nucleolus visible inside the nucleus. Top right corner: schematic representation of the structures.
If, at the end of the count, the results of the junior researchers’ did not match with those of the seniors, a double check was performed by the senior researcher to confirm the observed data (Table 1); the double-blinded method was used only in the first counting round; further, when the count was done, all researchers were made aware of the specimen’s details (number and patient name) to allow the comparison of the collected results.

Causes of death, post-mortem time of bone removal, and patients’ age were analyzed and eventually correlated with nuclei and nucleoli counting.

Based on the identification of the prevalent structure (nucleus or nucleolus), TBs were divided into two groups: (a) group 1 included the TBs in which nucleoli were identified more frequently than nuclei with a prevalence of 55% in the slide, and (b) group 2 included the TBs with a prevalence of nuclei over nucleoli of >55%. Such groups were analyzed to assess if differences in individual patient or specimen preparation characteristics were present between the groups.

**Statistical analysis:** It was performed using STATA® (Statacorp, College Station, TX 77845, Stati Uniti). Percentage was calculated, and mean and standard deviation were identified for numerical values. One-way ANOVA was used to compare the variance between the results obtained by the researchers. Nucleoli count for each segment (I, II, III, and IV) from both groups of researchers were evaluated to understand if there was a variance. Two-tailed t-test was used to evaluate the difference between nucleoli and nuclei identification in the count method. Spearman’s test was used to identify the correlation between the method used and presence of nucleoli; time of bone removal (<12 h or >12 h) and nucleoli identification; sex and nucleoli identification and age and nucleoli presence. Chi-square test was used to evaluate if the causes of death may determine differences in nucleoli identification. A \( p<0.05 \) was considered statistically significant.

**Table 1.** The table is an example of the data collection and shows the identification of nucleoli (1) or nuclei (0) in the different segment of human cochlea. When the researcher reported a doubt result, the data was collected as 0/1. In the last column on the right the general identification of nucleoli or nuclei by considering the TB in toto. In case of presence of nucleoli (or nuclei) in at least 3 on 4 segments of TB we considered an overall of nucleoli (or nuclei).

<table>
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Segment I: First Segment of Rosental Canal
Segment II: Second Segment of Rosental Canal
Segment III: Third segment of Rosental Canal
Segment IV: Fourth segment of Rosental Canal
RESULTS

Nucleoli versus Nuclei Count
Nuclei were identified in all cases (100%), whereas nucleoli were identified in 61% (75/123) of cases (t-test p<0.005). When sorting by the number of RC segments (3/4, 4/4), 83.7% (103/123) of nuclei and 92% (69/75) of nucleoli were identified in 4/4 segments (t-test: p<0.001), whereas 16.3% (20/123) of nuclei and 8% (6/75) of nucleoli were observed in 3/4 of segments (Figure 3).

Differences between junior and senior researchers were observed in nucleoli count. Junior investigators requested a double check by senior researchers in 18.7% of cases to confirm the identification of nucleoli: in 11.4% of cases for segment I (n=14), 1.6% for segment II (n=2), 1.6% for segment III (n=2), and 4.1% for segment IV (n=7). The senior researcher group had no doubts in nucleoli identification in any segment analysis. The difference between the results obtained by junior and senior researchers was statistically significant only for segment I (ANOVA: p=0.03), whereas non-significant difference was found for segment II (ANOVA: p=1), segment III (ANOVA: p=0.5), and segment IV (ANOVA: p=0.7). Considering the overall (segments I–IV) nucleoli count for each cochlea, the difference between junior and senior researchers was not statistically significant (ANOVA p=0.5). For identifying nucleoli, 20x magnification was used in 89.4% of cases (n=110), whereas 40x magnification was necessary in 10.6% of cases (n=13).

Specimen Characteristics
No significant correlation was found between decalcification methods used (TCA or EDTA) and the capacity to identify nucleoli (Spearman: P=0.9).

The coloration method was always the same, but the operator changed during the years, leading to a variation related to human bias. Post-mortem time of bone removal was <12 hours in the nucleoli group (mean: 5.9; SD: 2.4) in 54% of cases, whereas it was <12 hours in 47% the nuclei group (mean: 7.8; SD: 2.8). Post-mortem time was equally distributed between the two groups (t-test: p >0.05), which made our sample homogeneous. A higher identification rate of nuclei and nucleoli was found in case of bone removal ≤12 h rather than longer post-mortem time (>12 h) (Spearman: p<0.001).

Individual Characteristics of Subjects
In this study, the authors included 123 TBs extracted from 67 adults with a mean age of 69 years (range: 19–92) (Figure 4). The variation in the expected number of TBs (134) was due to the limited availability of both TBs from the same subject in some cases, in fact sometimes only a single TB was available.

The age was not statistically correlated with the visibility and the identification of nucleoli (Spearman: p=0.24).

Sample was equally divided with respect to sex (36 men: 52%; 33 women: 48%). Female sex was statistically correlated with the visibility and identification of nucleoli (Spearman: p=0.01).
Figure 4. Age distribution in the overall sample: the yellow bars indicate the number of subjects with age <60 years, and the blue the subjects with age >60 years. The subjects were divided by decades to facilitate the understanding of sample age distribution.

Figure 5. The image shows the overall number of temporal bones and the distribution of the different causes of death. Cardiovascular diseases (red bar) and cancer (grey bar) were the most common causes of death.

Figure 6. Pink tissue coloration (hematoxylin-eosin staining). Magnification 20×. No nucleoli are detectable.
Cardiovascular diseases were the most common cause of death in group 2 (35%), followed by cancer (30%) and infection (19%). In group 1, cancer (35%) and cardiovascular diseases (35%) were the most common causes of death, followed by infections in 14% of cases (Figure 5).

No significant correlation was found between the different causes of death and the nucleolus identification ($\chi; p=0.7$)

**DISCUSSION**

**Main Difficulties in Nucleoli Identification**

Our study shows no statistically significant differences in counting nucleoli or nuclei between expert and junior researchers; however, in 18.7% of cases, junior researchers requested a double check by seniors to confirm the identification of nucleoli. The nuclei identification and count did not show percentage of doubt because of the larger size that simplifies the identification [29, 30, 34] in particular by the junior researchers.

The bigger size of nuclei allows their identification on lower magnification compared with that necessary for nucleoli, and researchers did not need to change magnification during the count, thereby simplifying and speeding up the count process.

Alternatively, in this study, nucleoli identification was sometimes extremely difficult for younger researchers; however, nucleoli were always visible even when the nucleoli were identified (100%). Because of the small volume of the nucleoli and their different position on the specimens (higher or lower in the section), it was often necessary to change microscope focus to identify them, and eventually use a 40x magnification. Such magnification could explain why nucleoli were slightly more identifiable in all four segments compared to nuclei (92% versus 83.7%). Furthermore, we considered the presence of nucleoli as on/off. Therefore, when nuclei were identified in all four segments (83.7%), it was understood that the remaining structures were nucleoli. It is also relevant to state that every time nucleoli were observed, the nuclei were also identifiable, meaning that nucleoli were always observable in all four segments in 100% of TBs.

**Differences between Junior and Senior Researchers**

The junior researcher group required a double check from senior investigators to identify nucleoli, especially in the basal (segment I) and apical (segment IV) turn of the cochlea; this was probably related to the bone conformation of these segments which makes nucleoli identification more difficult in such areas. The senior researchers never showed doubts in identifying the nucleoli. This confirms the hypothesis that experience is the most relevant element in such analysis. Our data reported an overall identification rate of 61% for nucleoli and 100% for nuclei in the observed specimens. However, the statistical analysis comparing the results obtained by the researchers showed no significant difference in this count.

**Variables Associated with TBs Preparation**

The analysis of the methodology (fixation and decalcification) used to prepare TBs showed no significant influence of TBs preparation methods on nucleoli/nuclei identification rate, supporting the idea that this factor is not relevant to make nucleoli identification easier. The only parameter able to modify nucleoli identification rate in TB preparation technique was bone removal within 12 h after death. This agrees with the study by Kujawa et al. [15] who attributed such result to the oxidative phenomena acting on human tissue and inducing its deterioration along with increasing post-mortem time [22, 33].

**Variables Associated with Patients’ Characteristics**

Specific human tissue features, such as tissue acidity, may affect color absorption and therefore, nucleoli identification on histologic examination.

Our results show that it was easier to identify nucleoli in women than in men. We speculate that this could be correlated to the difference in food habits between women and men [36]. In fact, women prefer fruits and vegetables, which have an acid pH. This habit may affect the systemic pH concentration [37] and may explain the reason why nucleoli were more detectable in women than in men.

The acid-base reaction is also affected by protein concentration: some proteins are acidic as aspartate for example; therefore, an increase in protein concentration can explain the variation (more intense on hematoxylin reaction) in nucleoli coloration [38, 39]. Stan et al. [31] showed that RNA integrity is the best indicator of human tissue preservation. This parameter overlaps protein concentration and affects pH, and consequently, tissue coloration. Gadalaeta et al. [40] showed a decrease in protein concentration in nuclear structures during the aging process [40], although we did not observe any correlation between nucleoli identification rate and patients’ age.

Nicolas et al. [41] and Orsolic et al [42] also showed that cancer can increase protein concentration within nucleoli. Our sample showed that a high number of subjects died of cancer (70%), although the percentage of death caused by cancer was similar in both groups (nucleoli and nuclei). Furthermore, we did not find any significant difference in death causes between nucleoli and nuclei groups. We believe this can be related to the limited number of subjects involved in this study, which did not reach a sufficient power for statistical analysis.

Another factor potentially affecting nucleoli/nuclei coloration is the operator: eosin concentration may differ among various technicians, which can determine a change in color intensity from pink to violet. In the violet coloration, nucleoli appear darker than nuclei, which makes them visible on a low-magnification microscope as well. When the coloration is blue, nucleoli identification is more difficult, thus requiring a higher magnification (Figure 6). The standardization of preparation techniques should be underlined to make the results of different studies comparable.

**Study Limitations**

The lack of standardization in the TB preparation may have introduced a bias in nuclei/nucleoli counting procedure of our study. In particular, HE coloration has some limitations that are operator dependent: (a) the quantity of eosin varies and depends on the operator’s experience and habits; (b) variations of hematoxylin concentration can modify color nuances from blue to violet, thus affecting nuclei/nucleoli identification. The same is valid for the decalcification method that may be affected by subject characteristics and operator experience and habits.
CONCLUSION
The identification of nucleoli in human TBs may be more difficult than nuclei because of the difference in coloration and size of these structures. In this study, nucleoli identification was possible in all the cases. Because of the risk of cell double-counting, the correction factor suggested by Schuknecht et al. [28] and Nadol et al. [30, 32] should be applied to determine the correct number of SG cells when using the nucleoli identification procedure. Based on the results of this study, we suggest using nuclei count because of their easier recognition in all tissue conditions, especially when the researcher has a limited experience.

Ethics Committee Approval: The study was hosted at MEEI and because it was done on previously collected human temporal bones present in the Temporal Bone Bank of the Otopathology Laboratory, no ethics committee approval was necessary.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.


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Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES
Atraumatic Scala Tympani Cochleostomy; Resolution of the Dilemma

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OBJECTIVES: While an accurate placement in cochleostomy is critical to ensure appropriate insertion of the cochlear implant (CI) electrode into the scala tympani (ST), the choice of preferred cochleostomy sites widely varied among experienced surgeons. We present a novel technique for precise yet readily applicable localization of the optimum site for performing ST cochleostomy.

MATERIAL and METHODS: Twenty fresh frozen temporal bones were dissected using the mastoidectomy-posterior tympanotomy approach. Based on the facial nerve and the margins of the round window membrane (RWM), the cochleostomy site was chosen to insert the electrode into the ST while preserving the surrounding intracochlear structures.

RESULTS: There is a limited safe area suitable for the ST implantation in the area inferior and anterior to the RWM. There is a higher risk of scala vestibuli (SV) insertion anterior to that area. Posterior to that area, the cochlear aqueduct (CA) and inferior cochlear vein (ICV) are liable for the injury.

CONCLUSION: For atraumatic CI, precise and easy localization of the site of cochleostomy play a pivotal role in preserving intracochlear structures. Accurate setting of the vertical and horizontal orientations is mandatory before choosing the site of cochleostomy. The facial nerve and the margins of the RWM offer a very helpful clue for such localization; meanwhile, it is readily identifiable in the surgical field.

KEYWORDS: Cochlear implantation, cochleostomy, scala tympani, hearing preservation, residual hearing

INTRODUCTION
As the first clinically available artificial sensory organ in medicine, cochlear implants (CIs) represent a significant 20th-century surgical innovation [1]. It is mainly used to (re)habilitate patients with profound or severe hearing impairment who could not gain benefit from hearing aids [2]. Previously, assuming that ipsilateral hearing was compromised during surgery, implanting patients with residual hearing was prevented. However, improving the implants by refining the surgical procedures allowed the indication criteria to include many patients with substantial residual hearing [3, 4]. Soft CIs were introduced to preserve residual hearing during CI where scala tympani (ST) cochleostomy was described [5]. While an accurate placement of the cochleostomy is critical to ensure ST insertion, the choice of preferred cochleostomy sites varies widely among experienced surgeons [6]. We present a novel technique for precise yet readily applicable localization of the optimum site for performing ST cochleostomy.

MATERIALS and METHODS
With the approval of the Institutional Review Board in Mansoura Faculty of Medicine, a dissection study of 20 (7 left and 13 right) fresh-frozen temporal bones was performed by the first author with the aim of performing atraumatic ST cochleostomy. The dissection was initiated with an intact wall mastoidectomy followed by posterior tympanotomy (PT) with skeletonization of the chorda tympani (ChT) and the mastoid segment of the facial nerve (F.N.m), thereby achieving the maximum possible width for a more convenient evaluation of the target area of the cochleostomy. In addition, when the skeletonized F.N.m lies exactly transversely across the surgical field, it forms a vertical reference for the precise judgment of the site of cochleostomy (Figure 1). Then, the true...
round window membrane (RWM) was exposed until its annulus by drilling the bony overhang and removing the false membrane if present. When the annulus was clearly seen, imaginary tangents to the anterior and inferior parts of the annulus were considered parallel (Y) and vertical (X) to the F.N.m, respectively (Figure 2). To lead to the ST, the proposed cochleostomy site must be inferior to the X line. In relation to the Y line, different sites were chosen in different samples to verify the eventuality of each site to open ST. Under ample irrigation, low-speed drilling was performed using a 1-mm diamond burr until the preserved endosteal layer was exposed (Figure 1a) for later opening using a micro pick. Through the cochleostomy, the site of the osseous spiral lamina (OSL) was evaluated to appreciate the scala reached using each cochleostomy position. Electrode insertion was then performed with minimal pressure until the stopper reached the cochleostomy edge. The mastoid cavity was sealed to stabilize the electrode during the subsequent steps. Then, the intracochlear scalar position of the electrode was verified after performing a canaloplasty, thereby removing the tympanic membrane and ossicles and eventually transcanal drilling of the basal cochlear turn. In two specimens, lateral temporal bone resection was performed to provide sufficient space for performing the cochlear drill-out. Two bony rings were preserved around the cochleostomy and RWM. The membranous labyrinth of the basal cochlear turn was preserved to be opened with a hook along its upper and lower margins, preserving the OSL. The relation of the OSL to the cochleostomy, RWM, and inserted electrode was then appreciated.

RESULTS

The first performed cochleostomy was intended to be inferior to the X line and anterior to the Y line, as described above. After removing the endosteum, the cochleostomy was found to purely lead to the scala vestibuli (SV) of the basal cochlear turn and superior to the OSL. Thereafter, a second cochleostomy was performed to open the ST. During drilling, the second cochleostomy seemed inferior to the RWM and led to the ST (Figure 1c). Upon revising the

Figure 1. a-d. The importance of proper orientation of the F.N.m. as seen during performing a right cochleostomy. This figure illustrates the importance of proper orientation of the mastoid segment of the facial nerve (F.N.m). It should lie exactly transversely across the surgical field, for accurate interpretation of the site of cochleostomy. (a) When the nerve was oblique across the field, misinterpretation of the site of the cochleostomy (Coch.) occurred, giving the impression of being anteroinferior to the round window membrane (RWM). Notice the preservation of the endosteal layer, to be later opened with a micro pick, not directly by the drill. (b) After proper transverse positioning of the nerve across the field, the site that seemed to be anteroinferior appears clearly now to be rather anterior to the RWM. N.B.: The rotation is obtained by editing the photo. (c) The endosteum was removed to discover that this cochleostomy site (Coch. 1) had led to the scala vestibuli supero-lateral to the osseous spiral lamina and basilar membrane (*). Then, a second cochleostomy (Coch. 2) was performed in an attempt to gain access to the scala tympani. During drilling, the cochleostomy seemed to be inferior to the RWM. (d) However, rotating the photo to have the facial nerve across the field demonstrated that the second cochleostomy lies anteroinferior to the RWM (rather than inferior).
dissection photos, the importance of the F.N.m was recognized as a vertical reference for localizing the cochleostomy site. Having the F.N.m accurately transversely crossing the field, the initial cochleostomy that seemed anteroinferior to the RWM appeared rather anterior (Figure 1b), and the second cochleostomy appeared anteroinferior rather than inferior (Figure 1d). Subsequently, transverse orientation of the F.N.m became a routine step before deciding the cochleostomy site.

In the second specimen, most of the cochleostomy was inferior to the X line and anterior to the Y line (Figure 3a). This cochleostomy equally led to the ST and SV, with the OSL and the basilar membrane (BM) bisecting the opening (Figure 3b).

In the third specimen, the cochleostomy was completely inferior to the X line and nearer but anterior to the Y line. It led mainly to the ST and partially to the SV (Figure 4). The OSL and BM crossed the upper part of the opening, indicating an improvement of the cochleostomy position, but further optimization is still required.

In the fourth specimen, the cochleostomy was inferior to the X line. The Y line formed a posterior tangent to the drilled cochleostomy. The cochleostomy purely led to the ST but immediately below and flush with the OSL and BM (Figure 5).

In the fifth specimen, approximately 80% of the 1-mm burr was anterior to the Y line, as that shown in Figure 2 (yellow fine-dashed circle). This relative posterior shift was translated into more separation between the cochleostomy and OSL. However, the proximity of the electrode array and OSL can potentially result in an insertional trauma; therefore, it was decided upon the next dissections that the cochleostomy site would be modified to further protect the OSL.

In the specimens 6–11 and 13–20, the cochleostomy site was located inferior to the X line and exactly centered on the Y lines, as that shown in Figure 2 (solid-line and green circle). The burr had to drill through the crista fenestra before reaching the endosteal layer. Using this site, ST was purely reached in all 14 specimens. Meanwhile, the OSL and BM were not seen through the cochleostomy (Figure 6), indicating that these structures were kept intact and sufficiently distant from the cochleostomy site and consequently the inserted electrode. The ST position of the electrode was confirmed after performing the cochlear drill-out procedure. No gross trauma to the intracochlear structure was detected.

In specimen 12, despite locating the cochleostomy exactly as that in the latter specimens, the electrode entered the SV. A cochlear drill-out showed steeply vertical and posteriorly located OSL and BM. A posterior enlargement of the cochleostomy was made to expose the ST postero-inferior to the OSL, thereby determining the ideal co-

Figure 2. The intermediate and safe-range cochleostomy. When the annulus of the right round window membrane (RWM) is exposed, imaginary tangents are considered touching the anterior and inferior parts of the annulus. Y line: the anterior tangent that is parallel to the mastoid segment of the facial nerve (F.N.m). X line: the inferior tangent that is vertical to the F.N.m. The X and Y lines divide the area anterior and inferior to the RWM into three areas: A, B, and C. Area A is the area anterior to the RWM, anterior to the Y line, and superior to the X line. Anterior cochleostomy shall lie in area A. Area B is the area anteroinferior to the RWM, inferior to the X line, and anterior to the Y line. Without the presence of a precise definition, cochleostomy in any part of area B can be designated as an anteroinferior cochleostomy. Area C is the area inferior to the RWM, inferior to the X line, and posterior to the Y line. Inferior cochleostomy shall be performed in area C. The green circle that is centered on the Y line and inferior to the X line marks the site of our recommended intermediate cochleostomy position. The term intermediate describes its interposition between the areas of the famous anteroinferior and inferior cochleostomies. The yellow fine-dashed circle represents the most anterior limit of the safe cochleostomy range, through which atraumatic scala tympani implantation can be performed, whereas the red coarse-dashed one represents the most posterior limit of that range. The dashed parabola represents the estimated course of the spiral ligament and osseous spiral lamina. Therefore, the area anterosuperior to this dashed parabola corresponds to the scala vestibuli, and the area postero-inferior to it corresponds to the scala tympani.

Figure 3. a, b. Left (traumatic) anteroinferior cochleostomy, leading to the scala tympani and the scala vestibuli. (a) Left anteroinferior cochleostomy. F.N.m: Facial nerve, mastoid segment. Coch.: Cochleostomy. (b) Cochleostomy led to the area of junction between the scala tympani and scala vestibuli. OSL: osseous spiral lamina
chleostomy site in this particular specimen (Figure 7a). Thereafter, a concern arose about the integrity of the cochlear aqueduct (CA) and inferior cochlear vein (ICV). Further, the area inferior to the RWM was drilled to explore the latter structures, which were found very close to the posterior margin of the posterior extension of the cochleostomy (Figure 7b).

**DISCUSSION**

It is widely accepted that hearing preservation CI requires atraumatic electrode insertion into the ST, which is associated with superior audioligic outcomes and better speech perception performance [7, 8, 9]. The two major techniques for electrode insertion into the cochlea

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**Figure 4.** Right (traumatic) anteroinferior cochleostomy, leading mainly to the scala tympani and partially to the scala vestibuli. The osseous spiral lamina (<) is seen in the anterior one-fourth of the cochleostomy. Notice that the narrow space between the facial and chorda tympani nerves, together with the posterior rotation of the cochlea, prevented simultaneous visualization of the round window and the cochleostomy.

**Figure 5.** Right anteroinferior cochleostomy, touching the Y line and leading to the scala tympani immediately under the osseous spiral lamina (*). F.N.m: facial nerve, mastoid segment. RWM: anteroinferior part of the true round window membrane; the rest of the membrane is hidden medial to the facial nerve.

**Figure 6.** Left intermediate cochleostomy, the green circle is centered on the Y line and inferior to the X line. The cochleostomy purely led to the scala tympani; the osseous spiral lamina is not seen through the lumen of cochleostomy. F.N.m: facial nerve, mastoid segment; RWM: round window membrane.

**Figure 7. a, b.** Frank inferior cochleostomy. (a) Posterior enlargement of the initial "intermediate cochleostomy" that was centered on the Y line, in an attempt to expose the scala tympani (ST) postero-inferior to the osseous spiral lamina (OSL) (*). (#) denotes the defect anterior to the OSL after drilling the initial cochleostomy, which resulted in scala vestibuli (SV) insertion. Notice the position of the red circle (marking the ideal cochleostomy site, frank inferior cochleostomy in this case), in relation to the Y line, touching the line posteriorly. In addition, notice the steep vertical orientation of the OSL. RW: round window, the membrane was removed. F.N.: facial nerve. (b) The same specimen after lateral temporal bone resection and cochlear drill-out viewed from posterosuperior-lateral view. The basal cochlear turn has been opened to show the OSL, ST, and SV, with preservation of a bony rim (*) around the Coch. The channels for the cochlear aqueduct and the inferior cochlear vein (Ch.) seem very close to the posterior margin of the posterior extension. FN: facial nerve; HC: hypotympanic cells
are the RW insertion and the cochleostomy approaches. The optimal approach for standard CI electrode insertion is highly debated \(^{10,11,12}\). Even among the advocates of the cochleostomy approach, the choice of preferred cochleostomy sites widely varies among experienced surgeons \(^{16}\). A survey of CI surgeons confirmed this inter-surgeon variability; even some experienced CI surgeons preferred a site superior to the RW \(^{13}\). As subtle differences in the cochleostomy site may change the destination of the electrode from the ST to SV, a recommendation of future studies accurately documenting the exact location of the cochleostomy was recently given \(^{14}\).

The variability in the cochleostomy site may be explained by using the RW niche as the landmark for the cochleostomy \(^{15}\). In addition, relating the site of cochleostomy to the RWM are broad descriptions not suitable for this microscopic technique, thus requiring more specification. Meanwhile, the selection of the cochleostomy site in relation to the RWM is liable to inter-surgeon diversity, particularly with different vectors of vision through the PT. In other words, for this microsurgical procedure, where merely each fraction of a millimeter makes a significant difference, precise objective and easily recognized surgical landmarks are lacking. We present a clear clinically applicable description for choosing the cochleostomy site, thereby increasing the chance of atraumatic ST implantation.

Surgical intervention during cochleostomy necessitates detailed submicroscopical knowledge of cochlear morphology \(^{16}\). Accordingly, cochlear drill-out was conducted after electrode insertion. Not only the electrode position could be verified but also the complex relationships among structures comprising the hook region of the cochlea could be thoroughly appreciated from a surgical perspective, which is a great advantage of this study. Direct visualization of this area cannot be achieved in life surgeries because of the tiny cochleostomy and the presence of the endolymph. Similarly, a histological sectioning of the temporal bones after experimental implantation lacks such benefit. Previous works presented valuable descriptions and diagrams of the anatomy of the hook region of the cochlea \(^{17-20}\). In addition to these efforts, our style of study (cochlear drill-out after electrode insertion) will help a CI surgeon to build a mental map from the surgical perspective to address this microscopic yet complex area, which is dealt with surgically but not actually explored visually.

The first specimen showed the importance of adjusting the vertical and horizontal references for accurate judgement of the site of cochleostomy. A CI surgeon inspects a small part of the bigger tympanic cavity through the PT, along a tilted visual vector from posterior-superior-laterally to anterior-inferior-medially. This peculiar position may affect the accurate interpretation of the relationships of the structures of surgical importance to each other. In this concern, some landmarks may offer the necessary directional clues with varying degrees of reliability: the patient head orientation, supramastoid crest, and relation of the oval and RNWs. We suggest the F.N.m as the vertical reference after radiological exclusion of any anomalous course. Skeletonizing the F.N.m will maximize the PT and counteract the not uncommon tendency of leaving a thick bone covering the F.N.m for its safety; this thick bone would shift the vector of vision and shaft of the burr more anteriorly, which invites the risk of SV insertion. Having the skeletonized F.N.m precisely transversely running across the field gives an accurate vertical reference. The failure to appreciate the exact orientations together with the oblique vector of vision will lead to the misinterpretation of the correct site to perform the ideal cochleostomy, resulting in SV insertion (Figure 1).

Besides illustrating the extreme importance of setting the orientations prior to choosing the cochleostomy site, the unintended mistake during dissecting the first specimen yielded some additional benefits. First, the cochleostomy that appeared anteroinferior to the RWM was essentially almost anterior to it. Cochleostomy in this site led to the SV insertion. In addition, cochlear drill-out of all specimens revealed that the areas anterior and superior to the RWM are related to the SV. Consequently, these areas are excluded as routes for the ST. Second, the falsely apparent anteroinferior cochleostomy may explain the higher incidence of the SV insertion reported with cochleostomy compared with that of the RW route \(^{17,21}\). Third, in accordance with Figure 1c and 1d, the inferior-site cochleostomy reported by Briggs and colleagues \(^{22}\) would be perceived as anteroinferior to the RWM, after correcting the obliquity of the F.N.m, which was evident in the figure they presented to show their preferred cochleostomy site.

After exclusion of the sites anterior and superior to the RWM as routes for the ST, as stated above and in accordance with earlier works \(^{17,23}\) the anteroinferior and inferior sites will then remain available for further analysis. While some authors broadly described cochleostomies inferior or anteroinferior to the RWM as most favorable, \(^{6}\) more chose the site anteroinferior to the RWM \(^{23-27}\). Fewer authors suggested the inferior site for cochleostomy, \(^{21,28,29}\) but the choice was argued due to the risks of damaging the CA and ICV \(^{17}\). The latter citations sample the wide variability in surgeon approaches to the basal cochlear turn, \(^{28}\) a debate that promotes searching for a standardized approach.

In the second specimen, the OSL bisected the opening, denoting cochleostomy malposition. Despite being fractions of a millimeter, the deflections required correction in both vertical (to lie inferior to the X line) and horizontal scales (should be more posterior).

In the third specimen, the cochleostomy, which was located below the X line and anterior but closer to the Y line, had led mainly to the ST and partially to the SV. Although the ST can still be implanted, the BM may rupture during electrode insertion. Consequently, a posterior shift of the cochleostomy site toward the Y line seemed appropriate.

In the fourth specimen, where the cochleostomy was slightly posteriorly shifted to touch the Y line, pure ST opening could be achieved. Despite the better positioning of the opening, concern about frictional trauma between the electrode bands and BM made it wise to further shift the cochleostomy site posteriorly.

The fifth specimen showed a pure ST opening; however, the close proximity of the electrode to the OSL may render it vulnerable to insertional trauma with changing the vector of insertion of the electrode or with more bulky electrodes. Therefore, to minimize the possibility of insertional trauma, a further posterior shift was decided.

In specimens 6–11 and 13–20, the cochleostomy site was performed inferior to the X line and centered on the Y line. This cochleostomy site was termed intermediate cochleostomy position (ICP) because it lied between the popular anteroinferior and inferior cochleostomy
positions. It seemed optimum for pure ST opening, meanwhile perfectly protecting the OSL and BM from both the traumas of cochleostomy drilling and electrode insertion. This site that was chosen in 14 bones appeared logical to be the appropriate site for the first five bones by virtue of the posterior shift of the site of drilling in relation to the Y line. In other words, this cochleostomy site would be ideal for 19 bones out of the 20 (95%).

Only specimen 12 did not follow the rule after drilling the ICP. Cochlear drill-out revealed the electrode in the SV after passing supero-lateral to a steep vertically oriented OSL and BM immediately anterior to the Y line (Figure 7a). This posteriorly shifted OSL augmented the lateral surface area of the SV at the expense of the ST, explaining the SV insertion of the electrode. Such orientation of the OSL and BM will significantly impact the choice of the cochleostomy site and the insertion vector of the electrode. The ideal vector of insertion in such cases is better directed rather inferomedially in relation to the vector of the basal cochlear turn. Having such importance, the orientation of the OSL and BM is better to be evaluated through preoperative radiology; future formulation of a specific protocol will be required for this purpose. However, earlier studies may offer some help [30]. In the same specimen 12, a further posterior extension of the initial cochleostomy led to the ST inferomedial to the OSL. The latter posterior extension is equivalent to what we can call Frank inferior cochleostomy (FIC), touching the Y line. FIC by definition would be inferior to the RW, completely posterior to the Y line, and obviously inferior to the X line. FIC seems logical to be ideal for ST insertion in all specimens because it will guarantee an atraumatic opening of the ST and inferomedial to the OSL, regardless of its orientation. However, some drawbacks prevented choosing this site as the recommended optimum cochleostomy site. The first concern is the integrity of the CA and ICV because a damage of the latter will affect the viability of the spiral ganglion cells, which will negatively impact the CI performance and ICV because a damage of the latter will affect the viability of the mum cochleostomy site. The first concern is the integrity of the CA drawbacks prevented choosing this site as the recommended optimum cochleostomy site. The first concern is the integrity of the CA and ICV because a damage of the latter will affect the viability of the spiral ganglion cells, which will negatively impact the CI performance [30]. In this specimen, drilling inferior to the RWM revealed that the canals for the CA and ICV were very closely related to the posterior extension or FIC. However, more anatomical studies are recommended to precisely determine the safe area available for drilling inferior to the RWM and posterior to the Y line. Secondly, drilling in this area requires more removal of bone in the limited chorda-facial angle, which is a challenging task. In addition, the more posterior the cochleostomy, the higher the risk to injure the F.N.m, particularly with the rotating burr shaft, a more likely sequela in the setting of the posteriorly rotated RW. Moreover, a high jugular bulb is more liable to trauma with more posterior drilling. Another concern is that a more posterior cochleostomy will shift the vector of electrode insertion toward the modiolus rather than following the lumen of the ST along the basal cochlear turn, inviting a higher incidence of intracochlear insertional trauma.

CONCLUSION

We would recommend the ICP as the site of choice for atraumatic ST implantation, although it had a less theoretical success rate (95%) in comparison with the theoretical (100%) success for the FIC. Supporting this recommendation are the high success rate (95%), technical feasibility, and easier preservation of the F.N.m, CA, and ICV. The FIC should be reserved for cochleae with the steep orientation of OSL and BM, which is shown on a preoperative radiologic evaluation. In various anatomic constraints, hindering drilling the cochleostomy in the ICP; for the purpose of flexibility and easier applicability, we define the “safe cochleostomy range.” This is the safe range of cochleostomy positions that can be relied upon for atraumatic ST implantation (Figure 2). This range is dependent on the Y line, which is the anterior tangent of the RWM, parallel to the F.N.m. The 1-mm burr can start drilling in any point between two positions anteriorly, where most (approximately 80%) of the burr lie anterior to the Y line, till posteriorly where a similar portion lie posterior to this line. More anteriorly, the safety of the OSL and the related structures is less guaranteed. Similarly, the integrity of the CA and ICV becomes questionable further posteriorly.

Finally, all CI surgeons should enrich their armamentarium to include mastering with ease the RW insertion and cochleostomy approaches, in addition to subtotal petrosectomy. Having these tools readily available in hands, a CI surgeon will be ready to adapt the approach to the patient and not vice versa.

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Review Board of Faculty of Medicine, Mansoura University (R/17.09.14).

Informed Consent: N/A.

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REFERENCES

INTRODUCTION
Localization is the ability of an individual to be in tune with the direction of the sound source. It provides us with a more natural and comfortable listening experience. Our localization ability also acts as an alerting device against danger. The human brain is highly sophisticated in its computations; i.e., once a sound is heard, our auditory system processes the stimuli by primarily locating the sound source and then collecting the information from other senses and previous reminiscences. Therefore, localization plays a considerable role by alerting to anticipate dangerous situations. However, it typically involves the use of two ears. Binaural normal hearing individuals are consistently accurate in localization, whereas for individuals with hearing loss, there is a reduction in the performance of localization, which might lead to several psycho-social aspects in life, including stress and isolation [1, 2]. The degree of the inability to localize the sound source increases in individuals who have normal hearing in one ear and hearing loss in the other ear. For such individuals, their cognitive ability also plays an important role [3]. Although the use of amplification may not always restore localization to a normal level, an appropriate amplification device might help an individual to localize to some extent. A review has reported poorer performance than normal even with an appropriate hearing aid, which may be poorer than unaided localization when tested at the same sensation level. The reason could be that natural interaural differences, in terms of time and frequency differences, which provide information on an individual’s ability to localize the sound source. This might vary depending on the audibility and amplification devices. Although these behavioral measures are available, the perceptual quality of localization cannot be obtained using these measures. This study aimed to develop a questionnaire for auditory localization.

OBJECTIVE: Localization plays an important role in identifying the source of the stimuli. Aural localization is based on the phase (period-related time), intensity level, and spectral differences between the sounds at each ear. Various behavioral measures are available to check the interaural level, time, and frequency differences, which provide information on an individual’s ability to localize the sound source. This might vary depending on the auditory and amplification devices. Although these behavioral measures are available, the perceptual quality of localization cannot be obtained using these measures. This study aimed to develop a questionnaire for auditory localization.

MATERIALS and METHODS: A questionnaire was prepared, the content validated, and administered on 120 individuals in the age range of 18–50 years who were divided into three different groups.

RESULTS: The results of the descriptive and item analysis revealed a significant difference between the groups, with group I showing better localization ability. No significant difference was observed between the groups II and III. The receiver operating curve and cut-off scores were obtained. Individuals with a score of <42.5 on the questionnaire have better or good localization ability. The area covered under the curve is 0.987; therefore, the sensitivity and specificity of the questionnaire is also high.

CONCLUSION: It can be concluded that this questionnaire is a simple, valid, and preliminary measure for the auditory localization ability of an individual.

KEYWORDS: Hearing aid, cut-off, localization ability

INTRODUCTION
Localization is the ability of an individual to be in tune with the direction of the sound source. It provides us with a more natural and comfortable listening experience. Our localization ability also acts as an alerting device against danger. The human brain is highly sophisticated in its computations; i.e., once a sound is heard, our auditory system processes the stimuli by primarily locating the sound source and then collecting the information from other senses and previous reminiscences. Therefore, localization plays a considerable role by alerting to anticipate dangerous situations. However, it typically involves the use of two ears. Binaural normal hearing individuals are consistently accurate in localization, whereas for individuals with hearing loss, there is a reduction in the performance of localization, which might lead to several psycho-social aspects in life, including stress and isolation [1, 2]. The degree of the inability to localize the sound source increases in individuals who have normal hearing in one ear and hearing loss in the other ear. For such individuals, their cognitive ability also plays an important role [3]. Although the use of amplification may not always restore localization to a normal level, an appropriate amplification device might help an individual to localize to some extent. A review has reported poorer performance than normal even with an appropriate hearing aid, which may be poorer than unaided localization when tested at the same sensation level. The reason could be that natural interaural differences, in terms of time and level, are difficult to be provided from the hearing aid even with wide dynamic range compression (WDRC) hearing aids [4].

The complex auditory system utilizes various acoustic cues to localize the sound source [5]. This includes a combination of cues to determine the source of a sound in space caused by the spatial separation of the ears on either side of the head and interaural level and timing differences (ILDs and ITDs, respectively). These cues help a normal auditory system to localize the sound source both horizontally and vertically. According to previous theories, ILD and ITDs serve as keys for azimuthal sound localization, and spectral shaping of the sound by the outer ear and torso are primary cues for altitude localization and front-back discrimination [5-7].

This study was presented at the 49th Indian Speech and Hearing Association Conference, 6-8 January 2017, Kolkata, India.

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Although these theories are conceptual and behaviorally measured, there are fewer studies on correlation between conceptual and perceptual facts (self-reports) on auditory localization abilities, which brings about the need for self-reporting questionnaire studies on auditory localization abilities to supplement the behavioral correlation. There are fewer questionnaire-based studies on auditory localization. The widely used and adapted ones is the Spatial and Qualities of Hearing Scale (SSQ), which contains 49 questions on speech perception in quiet conditions and special hearing abilities, along with localization tasks, and rating the quality of speech perceived on a scale of 0–10 [8]. The questionnaire ‘The Spatial Hearing Questionnaire’ (SHQ) was also reviewed, which comprises 29 questions with similar domains of questions as in SSQ but does not include questions on the quality of speech or music [8]. It has a rating scale from 0 to 100. Both these questionnaires have more weightage on spatial hearing and speech perception in noise and quiet situations but do not specifically focus on localization abilities alone. Further, the existing questionnaires have a wide range of rating scale to be scored which is difficult to adapt in the Indian context. Studies have reported that localization and speech in noise perception have a large contribution to the acceptance of using a hearing aid for an individual. Thus, there is a need for the development and standardization of a questionnaire that will precisely focus on auditory localization abilities of an individual in the Indian context. However, with respect to localization, there is dearth of literature on the Indian context to measure the localization ability of individuals. Although other measures can be used, the response scales are more tedious for the analysis of problems faced because of localization inability. Therefore, a simple and cost effective tool is required to measure the degree of problem encountered because of poor localization ability. The present study aimed to develop a questionnaire for auditory localization and to administer the developed questionnaire on individuals with normal hearing, those with hearing loss (binaural), and those using binaural hearing aids.

MATERIALS and METHODS

Participants (age range, 18–50 years) were divided into three groups. Group I comprised 60 individuals with normal hearing (mean age, 34.4 and SD, 15.9 years; male:female, 27:33), group II comprised 30 individuals with binaural moderate sensorineural hearing loss for the frequency range of 250 Hz–8 KHz (mean age, 40.4 and SD, 9.8 years; male:female, 19:11), and group III comprised 30 individuals with binaural moderate sensorineural hearing loss for the frequency range of 250 Hz–8 KHz and binaural behind-the-ear hearing aid users (mean age, 38.9 and SD, 16.9 years; male:female, 17:13). The mean pure tone threshold (PTA) and speech recognition scores were 45.3 dB (SD, 4.6 dB) and 48 dB (SD, 5 dB), respectively, for participants in group II and 48.3 dB (SD, 3.3 dB) and 52 dB (SD, 3 dB), respectively, for those in group III. Participants in groups II and III were included if there were no otological or neurological problems and if speech identification scores were ≥70% for participants in group II and aided speech recognition scores were ≥70% for those in group III.

All participants were explained the purpose and nature of the study, and written consent was individually taken. The study was conducted in two phases: phase I included the development and validation of the questionnaire and phase II included the administration of the developed questionnaire. This study adhered to the Ethical guidelines for bio-behavioral research involving human subjects.

Phase I
To develop the questionnaire, information was obtained from the SSQ and SHQ [8,9]. Few other relevant questions were also added by the researcher, with additional inputs from individuals with hearing loss with difficulty in localization. The developed questionnaire comprised questions on individuals’ localization in noisy and quiet situations, and psychological problems that might be encountered because of poor localization were also included. This questionnaire was evaluated by 10 experts in the field of audiology and 10 experts in the field of speech language pathology for content evaluation. Based on their suggestions, the questions were deleted or modified with 75% criteria of the average scores for each question. The modified final developed questionnaire comprised 22 questions with various subsections and was rated on a 5-point rating scale as “1: never, 2: almost never, 3: sometimes, 4: almost always, and 5: always”. The different sections included 15 questions on localization in noisy situations and 7 questions on localization in quiet situations. The 4 subsections under localization in noisy situations where traffic zone (5), outdoor (4) indoor, and near your locality situations (2), and psychological aspects (4) were also included (Annexure A).

Phase II
All participants were instructed on the procedure to rate the questionnaire. The questions in the questionnaire were orally presented either in English or in Kannada to the participants, and their responses were filled verbatim by researcher. The questionnaire was individually presented. Data obtained were tabulated and entered in the Statistical Package for Social Sciences (SPSS) software version 17 (SPSS Inc., Chicago, IL, USA).

RESULTS

The item analysis of the questionnaire was descriptively conducted. Shapiro-Wilk test of normality was administered. As the test reported non-normal distribution non-parametric test was done for further analysis of the obtained data. Table 1 presents the mean, standard deviation, and median for the raw scores of the item analysis for administered questionnaire on auditory localization abilities.

Table 1 shows that participants in group I procured the least scores of mean value ranging from 1.05 to 1.76 in all questions within the subsections than those in groups II and III, except for question A3 on traffic situations in the subsection A that showed the highest score of 2.15, which was suggestive of the difficulty faced by individuals with normal hearing in attending to speech in noise situations, particularly in traffic zones. The extent of difficulty faced by individuals with hearing loss and hearing aid users in traffic situations was evident by the observed highest scores of 3.63 for A3 in the traffic subsection by hearing aid users and a yet closer score of 3.41 by individuals with hearing loss in the same domain. Apart from the traffic subsection, the observed mean values of participants in group II in psychological effects subsection D had elevated scores of 3.68 for D3 than those in the other subsections, which indicated that individuals with hearing loss endure the problem of focusing on sound stimuli in noisy situations and also have the perception of losing concentration when the sound seem confusing because of the difficulty in localization. Further, the total scores indicated that, among the three groups, group I had lesser scores in all subsections of noisy and quiet situations, more principally had the least score of 4.43 for subsection D, and had
no psychological dilemma; the groups II and III incurred more scores of 25 and 28, respectively. However, the same trend was followed in all subsections.

Pearson's chi-square test of association was conducted to examine the association between groups of participants and the responses obtained. The results revealed a significant difference between the groups \( \chi^2(8) = 24.980, p<0.05 \) for all the responses to the questions in the questionnaire. Although differences were observed between the groups, the responses of participants in group I were superior than those of the other groups. A non-parametric Friedman test of differences among repeated measures was conducted for all groups of participants, which rendered a chi-square value of 44.086 with statistical significance (\( p<0.001 \)) for group I. The chi-square value of Friedman test for groups II and III were 0.378 and 1.054, respectively, which were not significant (\( p>0.05 \)). Pairwise analysis was performed within the subsection of the total scores for quiet and noise situations of the questionnaire using the Wilcoxon signed rank test for group I (Table 2).

Table 2 shows a significant difference between all scores of the subsections, except for the section A vs. B and C vs. D of participants in

<table>
<thead>
<tr>
<th>Questions</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Median</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>A. Traffic situation</td>
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<td></td>
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<tr>
<td>A1</td>
<td>1.20</td>
<td>1.00</td>
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<td>A2</td>
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<td>1.58</td>
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<tr>
<td>TA</td>
<td>7.86</td>
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<td>B. Outdoor situation</td>
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<tr>
<td>B1</td>
<td>1.70</td>
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</tr>
<tr>
<td>B2</td>
<td>1.31</td>
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<td>B3</td>
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<tr>
<td>B4</td>
<td>1.21</td>
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<tr>
<td>TB</td>
<td>5.91</td>
<td>6.00</td>
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<tr>
<td>C. Indoor situation</td>
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<tr>
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<tr>
<td>TQ</td>
<td>10.83</td>
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Note: A1–A5, B1–B4, C1–C2, D1–D4, and Q1–Q9 indicate the raw scores of each item of the questions in sections A, B, C, D, and Q of the questionnaire. TA, TB, TC, TD, and TQ indicate the total of the raw scores in the respective section of the questionnaire.
The results of Wilcoxon signed rank test for the total scores of the subsection of the response to the questionnaire for group I

<table>
<thead>
<tr>
<th>Subsections of the questionnaires</th>
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<th>p</th>
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<td>TD–TA</td>
<td>8.628</td>
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<tr>
<td>TC–TB</td>
<td>1.958</td>
<td>0.050</td>
</tr>
<tr>
<td>TD–TB</td>
<td>8.440</td>
<td>0.000</td>
</tr>
<tr>
<td>TD–TC</td>
<td>3.245</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: TA, TB, TC, and TD indicates the total raw scores of the subsections of the questionnaire on auditory localization abilities.

Table 3. Pairwise comparison across the subsection of questionnaire for all three groups

<table>
<thead>
<tr>
<th>Localization in quiet vs. noisy situations</th>
<th>Z</th>
<th>Asymp. Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.80</td>
<td>0.000</td>
</tr>
<tr>
<td>Group II</td>
<td>3.30</td>
<td>0.001</td>
</tr>
<tr>
<td>Group III</td>
<td>0.853</td>
<td>0.394</td>
</tr>
</tbody>
</table>

From the above figure the closer the curve of the blue that follows the left-hand border and then the top border of the ROC space, the more accurate the test. The area under the curve was 0.987. Considering the raw scores, the cut-off would be 42.5. Thus, if the total score of the questionnaire is <42.5, then it indicates that the individual has a good localization ability, whereas if the total scores are >42.5, then it indicates that the individual has difficulty in the localization of the sound source. Therefore, depending on the cut-off scores obtained by individuals, training regimens can be developed to enhance the sound localization performance in individuals with impaired localization abilities.

DISCUSSION

The results of the present study revealed that the ability to correctly localize sounds is an important feature of the auditory system, which is directly linked to the ability of binaural listening that is helpful in difficult listening situations, such as during noisy and reverberation situations. However, with regard to individuals with hearing loss, this information is diminished and the localization is poor. The reason that the participants in group I outperformed could be that these individuals could use ILDs, ITDs, and monaural spectral cues compared with individuals with impaired ears. Similar differences have been reported were the individuals with normal hearing have better localization than impaired use due to the better ILD, ITD and spectral cues of the signal through the measurement of degree and errors of localization [10]. Through the application of digital signal processing, WDRC and wireless transmission techniques in hearing aids the cues for localization are preserved to a certain extent. However, in the present study, the group III participants though they were hearing aid users the reported poorer ability for localization in the self rated questionnaire. There are also reports that DLI did not improve between unaided and aided conditions [11]. Aided localization is reported to be still poorer than that of individuals with normal hearing, and in some individuals, aided localization ability may be even poorer than unaided localization at the same sensation level [12]. This revealed that the cues for localization might be affected because of impairments but is difficult to cope even with hearing aids. Although there are improvement to be reported in measures of localizations due to training and other
factors, self satisfaction for localization might be lesser [13-15]. Therefore, a questionnaire in simpler form might help to probe in detail regarding different issues related to the poor localization ability and satisfaction in localizing the source in individuals with hearing loss.

CONCLUSION
The study provides a useful tool in understanding the degree of localization problems that are faced by individuals with hearing loss. Although, several individuals use hearing aids for a longer duration, they are still not completely satisfied with their localization ability in noisy situations. This questionnaire can be used as a simple tool to check for difficulties faced in localization and can further help in investigating possible upbringings to be implemented for better output resolutions in amplification devices, and tailoring these fine tunings in hearing aids would fetch more comfortable and lively experience in individuals using hearing aids. This questionnaire also serves as a simple tool to assess the pre- and post-outcome measures of the use of amplification device and can be used for measuring the outcomes of the auditory localization training.

Ethics Committee Approval: This study adhered to the “Ethical Guidelines for Bio-Behavioral Research Involving Human Subjects” set by All India Institute of Speech and Hearing Ethics Committee (Venkatesan, 2009).

Informed Consent: Written informed consent was obtained from the patient who participated in this study.

Peer-review: Externally peer-reviewed.


Acknowledgements: The authors would like to thank the Director, All India Institute of Speech and Hearing, Mysuru, for granting permission to carry out the study and the participants for their cooperation.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES
# ANNEXURE A

## Questionnaire on auditory localization ability

**Name:**  
**Age/Sex:**  
**Education:**  
**Phone no:**  
**Case number:**  
**PD:**  
**HA user:** Yes/No

**Instruction:** Read the questions and indicate your choice by ticking against the appropriate column.

### Localization in noisy situations

<table>
<thead>
<tr>
<th>A. Traffic zone</th>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Almost Always</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. When you are standing on the footpath of a busy street, do you have difficulty in telling from which direction or side is a bus or truck or any other vehicle coming from before you see it?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. When you are standing on the footpath of a busy street, do you have difficulty in telling how far away a bus or truck is by the sound alone?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. When you are driving a two wheeler wearing a helmet, do you have difficulty to hear what the other person sitting behind you is saying?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. When you are driving two wheeler wearing a helmet, do you have difficulty in finding from which side is the sound of an ambulance coming?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. When you are driving two wheeler (wearing a helmet) or a four wheeler (windows closed), do you have difficulty in finding from which side is the sound of another vehicle coming?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Outdoor situations</th>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Almost Always</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. You are outside. You can hear an airplane. Do you find it hard to tell where the plane is in the sky by the sound alone?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. You are outdoors in an unfamiliar place. You can hear the sound of a dog barking. You cannot see where it is. Do you find it hard to tell where it is without having a look?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. In the street, you can hear pedestrians walking. Do you find it difficult to judge the direction of sound by their footsteps alone?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Do you find it difficult to determine the location of a music source, say orchestra or a music band procession, when you cannot see it?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Localization in quiet situations

<table>
<thead>
<tr>
<th>C. Indoor and near your locality</th>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Almost Always</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. When you are watching TV at your house, if there is a bang of a window door due to wind, do you have difficulty in identifying which door is it?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. You are in a high-rise apartment or in the second floor of a building/balcony/bridge. You can hear sound from another floor or from the ground floor. Do you have difficulty in telling whether the sound is coming from above or below you?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D. Psychological aspects</th>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Almost Always</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you become nervous in a strange place due to localization difficulty?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Do you avoid busy areas, such as noisy areas?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Do you avoid shopping alone in markets outside?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Do you lose your concentration when the sound seems confusing?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Localization in quiet situations

<table>
<thead>
<tr>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Almost Always</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you turn the wrong way when someone that you cannot see calls out to you when in a quiet room?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. You are in an unfamiliar house. It is quiet. You hear a door slam. Do you have difficulty in identifying the door from which the sound came?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. You are sitting between two people. One of them starts speaking. Do you have difficulty to identify whether the person is on your left or your right without having to look?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Do you have difficulty to identify the location of a man’s voice when you cannot see him in a quiet room?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Do you have difficulty to identify a woman’s voice when you cannot see her in a quiet room?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Do you have difficulty to identify a child’s voice when you cannot see him/her in a quiet room?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. You are at home in a quiet room. There are other people in the house (friends or family). They are talking in another room and you can hear them. Do you have difficulty in telling which part of the house those people are in?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. You are in a quiet room and your mobile phone rings at a certain distance far from you. Do you have difficulty in easily reaching your phone by hearing the ringtone?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. You are at home and you hear the running water sound from an open tap in one of the rooms. Do you have difficulty in finding the leaking tap?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Satisfaction with Life among Mothers of Pediatric Cochlear Implant Candidates: The Impact of Implant Operation and Sociodemographic Factors

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Clinic of Child and Adolescent Psychiatry, Edirne Sultan I. Murat State Hospital, Edirne, Turkey (MAA)

OBJECTIVES: To evaluate the satisfaction with life among mothers of pediatric cochlear implant candidates regarding implant surgery and sociodemographic factors.

MATERIALS and METHODS: Mothers of 160 pediatric patients with profound sensorineural hearing loss who underwent unilateral cochlear implant surgery were included. A questionnaire form with items on sociodemographic-familial characteristics and Satisfaction with Life Scale (SWLS) was employed via face-to-face interview method before and 12 months after the implant surgery.

RESULTS: The SWLS scores significantly improved after the implant surgery [from 19.1 (7.0) to 28.9 (4.0), p<0.000]. Being unemployed vs. employed [17.9 (6.9) vs. 24.0 (5.3), p=0.000], having another child with hearing disability [13.5 (5.7) vs. 19.7 (6.9), p=0.001], younger (12–24 months) vs. older (>24 months) age of the child at the time of implant surgery [7.1 (0.4) vs. 19.7 (6.6), p=0.001], absence vs. presence of regular follow-up visits [13.0 (0.0) vs. 19.4 (7.1), p=0.002], and presence vs. absence of change in social life after the diagnosis of disease [17.3 (6.5) vs. 20.9 (7.1), p=0.001] were associated with significantly lower SWLS scores among mothers. SWLS scores were positively correlated with patient’s age at the time of implant surgery (r=0.206, p=0.009), whereas negatively correlated with the number of household members (r=−0.406, p=0.000) and number of children (r=−0.310, p=0.000).

CONCLUSION: In conclusion, our findings revealed the association of cochlear implantation with a significant increase in mother’s life satisfaction, despite the unemployment, presence of another child with hearing disability, and crowded household. Our findings emphasize on the consideration of family systems with special attention to mother’s emotional experiences and occupational competence in the intervention programs.

KEYWORDS: Pediatric sensorineural hearing loss, cochlear implant, maternal life satisfaction, occupation, emotional experience

INTRODUCTION

Cochlear implants have become the standard of care in the management of children with severe to profound sensorineural hearing loss [1]. Along with improved technical performance of the device in providing hearing sensitivity within the speech, there has been an expanded patient candidacy and progressive reduction in the minimum age for implantation over the years [1].

Cochlear implantation has been associated with improved hearing as well as speech and language skills and better academic performance in implanted children [1]. However, the parental involvement and consideration of family perspective in the management of the disease are considered crucial in the follow-up and attainment of desirable language and reading skills among cochlear implanted children [2-6].

Early childhood hearing loss has been associated with unique and long-term challenges for parents in terms of communication difficulties, medical care, and academic problems [6, 7]. Thus, parenting a child with hearing disability is accompanied with increased stress levels among both parents, where mothers are considered to be particularly prone to increased stress due to high level of responsibility in attending appointments, managing hearing devices, and provision of home care and therefore
considered to develop different ways of coping strategies as compared to fathers [7, 8-10].

Parental involvement and consideration of family perspective in rehabilitation and family-oriented interventions are of utmost importance in the management of cochlear implanted children [8, 11]. However, majority of past studies among cochlear implanted children have concentrated on the efficacy of the procedure in terms of speech perception and production with limited data on the outcomes from the broader perspectives, including the role of family, the effect of hearing loss on the family, and the needs and perspectives of parents during rehabilitation [11-13].

This study was therefore designed to evaluate satisfaction with life among mothers of pediatric cochlear implant candidates regarding implant surgery and sociodemographic factors.

**MATERIAL and METHODS**

**Study Population**

Mothers of 210 pediatric patients with profound sensorineural hearing loss who underwent unilateral cochlear implant surgery between 2010-2014 years in a tertiary care clinic were initially enrolled in this study. Patients aged >18 years with co-morbid disabilities, parental hearing loss, parental divorce, or non-parent custody were excluded from the study. Accordingly, due to exclusion of 50 patients or mothers that met the exclusion criteria, the final study population comprised 160 mothers [mean (standard deviation, SD) age: 29.5 (5.5) years].

**Assessments**

A questionnaire form with items on sociodemographic-familial characteristics and Satisfaction with Life Scale (SWLS) was employed via face-to-face interview method for each mother before the implant surgery. SWLS was applied once again to each mother 12 month after the cochlear implant surgery.

**Sociodemographic Questionnaire Form**

Sociodemographic questionnaire form included items on maternal characteristics (age, educational status, and occupation), familial characteristics (number of household members, income level, health insurance, number of children, other children with hearing loss, and consanguineous marriage), patient characteristics (age, sex, age at cochlear implant implementation, duration of hearing device prior to cochlear implant, and number of annual hospital visits) and burden of the disease, including overall impact (occupational problems, physical tiredness, need for support, treatment cost, transport problems, and problems with care of other children), and specific impacts on family life (interpersonal relations, individual responsibilities, and stress), social life (change in social environment, vacations, entertainment activities, and relations with friends), and emotional life (loneliness, panic, guilt, fear, and inhibition of negative feelings).

**Satisfaction with Life Scale**

SWLS is a short 5-item instrument developed by Diener et al. [14] in 1985 as a tool for the measurement of the life satisfaction component of subjective well-being. It is based on a 7-point Likert style response scale (1: strongly disagree to 7: strongly agree) with the items summed to provide a final score ranging from 5 (minimum life satisfaction) to 35 (maximum life satisfaction). Adaptation and validation of the Turkish version of the SWLS was performed by Durak et al. [15] in 2010.

**Statistical Analysis**

Statistical analysis was performed using The Statistical Package for the Social Sciences for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). Numerical data were analyzed using the Mann–Whitney U-test, whereas change over time was evaluated using the Wilcoxon test. Correlation analysis was performed via Spearman correlation analysis. Data were expressed as “mean (SD);” minimum-maximum, and percentage (%) where appropriate. p<0.05 was considered statistically significant.

**RESULTS**

Maternal, family, and patient characteristics:

The mean age of the mothers in this study population was 29.5±5.5 years. Most of them were primary school graduates (59.0%) and unemployed (80.0%) with low levels of monthly income (85%) (Table 1).

The mean number of overall family members and children within families were 4.2 and 1.6 respectively, whereas consanguineous marriage and another child with hearing loss were present in 25.0% and 10.0% of families, respectively (Table 1).

The mean age of the pediatric patients was 5.8±2.2 (range, 3–13) years; 70% were girls, and 70% were diagnosed with hearing loss before 12 months of age (40% at 0–6 months of age) and 70% were using the hearing device before 24 months of age. The age at implant surgery was 36–48 months in 50.0%, 24–36 months in 25.0%, and 48–60 months in 20.0% of patients, whereas only 5% of patients were younger than 24 months (Table 1). All patients had unilateral cochlear implants along with a contralateral hearing aid.

Regular follow-up was evident in majority of patients (95%) based on visits (5–7 times) per year (60.0%) (Table 1).

Burden of the disease:

The most significant burden of the disease was the treatment cost (30.0%), followed by work-related problems (25.0%) and problems with the care of other children (15.0%) (Table 2).

Considering the impact of disease on family life, 70.0% of the mothers identified an increase in either individual responsibilities or stress in the family, whereas 30.0% indicated improved interpersonal relations and connection in the family after the diagnosis of disease (Table 2).

Change in social life after the diagnosis of the disease was evident in 50.0% of the mothers, including new social environment (100.0%) with a change in entertainment activities (60.0%), relations with friends (30.0%), and vacation routine (10.0%) (Table 2).

The most predominant emotions experienced by the mothers were loneliness (44.0%), inhibition of negative affection (20.0%), and guilt (19.0%), followed by fear (15.0%) (Table 2).
SWLS scores with respect to implant surgery and study variables:

Mean±SD SWLS scores of mothers significantly improved after the implant surgery from preoperative values of 19.1±7.0 to 28.9±4.0 after 12 months of surgery (p<0.000) (Table 3).

Being unemployed vs. employed (p=0.000), having another child with hearing disability (p=0.001), younger (12–24 months) vs. older (>24 months) age of child at the time of implant surgery (p=0.001), absence vs. presence of regular follow-up visits (p=0.002), and presence vs. absence of change in social life after the diagnosis of disease (p=0.001) were associated with significantly lower mean (SD) SWLS scores among mothers indicating poorer satisfaction with life. Sex of the patients had no significant impact on mother’s SWLS scores (Table 3).

SWLS scores were positively correlated with patient’s age at the time of implant surgery (r=0.206, p=0.009), whereas negatively correlated with the number of household members (r=−0.406, p=0.000) and the number of children (r=−0.310, p=0.000) (Table 3).
DISCUSSION

Our findings among the mothers of pediatric cochlear implant candidates with profound sensorineural hearing loss revealed significantly improved maternal life satisfaction after the cochlear implant surgery. However, unemployment, having another child with hearing disability, having their children implanted before 24 months of age, crowded household, lack of attention to follow-up, and change in maternal social life due to the disease were the negative factors associated with significantly lower SLWS scores and thus results in poorer maternal life satisfaction.

Cochlear implant surgery was associated with significantly improved postoperative SWLS scores in mothers within 12 months of surgery as compared with the preoperative scores in our cohort. This seems consistent with the heightened levels of stress reported to be experienced by parents of cochlear “non-implanted” vs. cochlear “implanted” deaf children and the significant decrease in the intensity of stress experienced by the parents after the cochlear implant surgery, even to levels comparable to those of the parents of a child with normal hearing ability [16].

Similarly, in a past study among the mothers of cochlear implanted children, cochlear implantation was reported to be associated with a decrease in the level of maternal depression, anxiety, and stress and emphasized to have a potential to improve maternal psychological parameters [6].

Nonetheless, it should be noted that the parents of cochlear implanted children were reported to experience higher levels of stress and poorer psychological adjustment than those of normally hearing children [10, 17, 18].

In a past study in Turkey among 161 cochlear implanted pediatric patients, preoperative period and first week after surgery were considered to be extremely stressful by 93.1% of the parents, whereas 93.2% stated that they were relaxed after realization of the first response of their child to their voice [3]. Continued parental stress after the surgery has been considered to be associated with ongoing demands of the children as well as a gradual increase in communication and behavior problems [9].

Child’s age at the time of implantation is considered to have a significant inverse impact on the postoperative outcomes, with better outcomes expected for those with younger age [19]. Several studies reported better performance among children implanted before 2 years of age than those implanted at older ages [1, 20, 21]. Hence, preoperative poorer life satisfaction among the mothers of children implanted “before” versus “after” 2 years of age in our cohort seems likely to be related to experiencing an unnecessarily high sense of urgency, anxiety, and pressure about accessing the implant option in the preoperative period [13]. Nonetheless, given the suggestion of a quicker and more complete adaptation to a cochlear implant in case of earlier interventions [22], their life satisfaction scores seem to markedly improve in the postoperative period provided their expectations are realistic regarding their child’s condition [13].

Although developments in newborn hearing screening led to an earlier and easier diagnosis of hearing loss with a reduction in the age at diagnosis, amplification, and intervention, past studies indicated that a considerable delay exists between the age at identification of hearing loss and age at cochlear implantation [23, 24]. Parent’s low level of awareness regarding cochlear implantation, low educational level, and lack of financial resources were suggested among the possible reasons for delays in performing cochlear implantation [23, 24]. In our study, while 40% of children were diagnosed before 6 months of age and 70% before 12 months of age, only 30% were using the hear-

<table>
<thead>
<tr>
<th>Table 3. Satisfaction with Life Scale (SWLS) scores with respect to implant surgery and study variables</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Satisfaction with Life Scale score</strong></td>
</tr>
<tr>
<td><strong>Cochlear implant</strong></td>
</tr>
<tr>
<td>Before implant surgery</td>
</tr>
<tr>
<td>After implant surgery</td>
</tr>
<tr>
<td><strong>Gender of the patient</strong></td>
</tr>
<tr>
<td>Girl</td>
</tr>
<tr>
<td>Boy</td>
</tr>
<tr>
<td><strong>Age at implant surgery</strong></td>
</tr>
<tr>
<td>12-24 months</td>
</tr>
<tr>
<td>&gt;24 months</td>
</tr>
<tr>
<td><strong>Employment</strong></td>
</tr>
<tr>
<td>Employed</td>
</tr>
<tr>
<td>Unemployed</td>
</tr>
<tr>
<td><strong>Another child with hearing loss</strong></td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td><strong>Consanguineous marriage</strong></td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td><strong>Regular annual follow up</strong></td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td><strong>Change in social life</strong></td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td><strong>Correlations</strong></td>
</tr>
<tr>
<td>Patient age</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Maternal age</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>#of household members</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>#of children</td>
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<td></td>
</tr>
</tbody>
</table>

Min: minimum; max: maximum; SD: standard deviation; #: number
ing device at 12 months of age and only 5% were using the cochlear implant at 24 months of age. Likewise, in a past study from Turkey, low level of parental knowledge and low socioeconomic status were considered to be responsible for the delay in identification of hearing loss and the increase in the time lapse between amplification and intervention [26].

In fact, among multiple factors affecting developmental outcomes of children with hearing impairment, the effect of timing of intervention is considered the minimal as compared with other demographic factors related to the child and family [13, 27].

Indeed, parent’s daily problems in relation to raising deaf children (socialization, habilitation demands, and parenting role, financial difficulties, services) were reported to be correlated with stress levels and life satisfaction [28]. Authors also noted that spouses and other parents were identified as partners for the collaborative daily problem solving and coping by parents of deaf children [28]. Notably, despite increased individual responsibilities and increased stress in the family environment after the diagnosis of disease, mothers in our cohort also identified improved interpersonal relations and connection within the family.

Moreover, our findings are also in line with the feelings of guilt and tiredness related to the increased responsibility of being a teacher or trainer alongside a mother/father of the child, and thus concerns about performance failure reported among parents of children with hearing disabilities [11, 28]. These feelings have also been suggested to result in joining support groups comprising other parents with hearing-impaired children or connecting with associations and community centers [29, 30].

Mothers in our cohort identified occupational life, care of other children, and increased individual responsibilities as the most problematic areas of parenting a child with a hearing disability. This supports the statement that parents, especially mothers, felt responsible for their child's outcomes and take many roles and are at risk of experiencing negative emotions, such as guilt and unhappiness, in case of poor outcomes [2, 31, 32].

Consistent with the 80% unemployment rate in our cohort, having children with disabilities was reported to cause some parents to quit their job leading to significant financial problems for the families [11, 33]. Indeed, being unemployed, the presence of a crowded household, the number of children, and disease-related changes in social life were the factors associated with a poor maternal life satisfaction in our cohort. This supports the reported relation of employment status, occupational competence, role overload, number of children, and social support to health and life satisfaction of mothers [34].

Hence, our findings emphasize the importance of implementing family-centered intervention programs with close attention to the emotional experiences of mothers, the availability of the support networks, and the level of occupational competence alongside consideration of efforts to enhance family relationships or increase awareness and perceptions of the available support [2, 17, 34].

Notably, incorporating parents as inalienable members in the rehabilitation process and considering individual needs and family dynamics via persistent and long-term multi-dimensional auditory-verbal intervention programs and counseling were shown to be associated with positive effects in a past study among hearing-impaired children's parents in Turkey [31].

Additionally, problems with transportation to the implantation center, lack of access to special services as well as travel and commuting costs to the rehabilitation centers were identified among the significant problems experienced by families with cochlear implanted children, including those from Turkey [3, 11, 35]. This seems notable given that timely detection and action in hearing disorders necessitates access to high-quality screening and diagnosis services in the area [11].

CONCLUSION

In conclusion, our findings revealed the association of cochlear implantation with a significant increase in the mother's life satisfaction, despite sociodemographic factors with a negative impact on life satisfaction such as unemployment, the presence of another child with hearing disability, crowded household, and change in social environment. Hence, our findings emphasize the increased parental awareness about the role of earlier provision of a cochlear implant in terms of not only improved hearing outcomes in the children but also improved maternal life satisfaction, provided that maternal expectations are realistic in relation to their individual child's condition. Our findings also emphasize on the consideration of family systems with special attention to the mother's emotional experiences and occupational competence along with the availability and awareness of support networks in the family-centered intervention programs.

Ethics Committee Approval: The study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee (Date/Protocol No: 2012/13).

Informed Consent: Written informed consent was obtained from each mother following a detailed explanation of the objectives and protocol of the study.


Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES


Original Article

Effect of Body Mass Index on Middle Ear Resonance Frequency

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OBJECTIVE: Multifrequency tympanometry (MFT) analyzes tympanograms obtained using different probe tones between 226 and 2000 Hz. An important parameter of MFT is resonance frequency (RF). Studies have recently demonstrated that the RF value can vary depending on many factors. To provide new data regarding MFT, middle ear RF values were investigated with regard to body mass index (BMI).

MATERIALS and METHODS: This study included 78 volunteers (i.e., 156 ears) aged 18-40 years who did not have hearing loss and whose otoscopic examinations were normal. Hearing thresholds were measured using pure tone audiometry, and RF values were recorded with immittance measurements. The participants were divided into the following three groups according to their BMI: <18.5 kg/m², Group 1; 18.5-24.9 kg/m², Group 2; and >25 kg/m², Group 3. The RF values were also analyzed.

RESULTS: Although there was no significant difference between Groups 1 and 2 in terms of RF values, a significant difference was observed between Groups 1 and 3 and between Groups 2 and 3.

CONCLUSION: In the light of these data, BMI values should be considered when middle ear RF values are assessed.

KEYWORDS: Body mass index, multifrequency tympanometry, resonance frequency

INTRODUCTION

Multifrequency tympanometry (MFT) consist of the analysis of tympanograms of different probe tones between 226 and 2000 Hz and measures individual vectors of complex admittance (Y), namely susceptance (B) and conductance (G) [1]. An important parameter of MFT is resonance frequency (RF). The middle ear transmission system comprises the mass and stiffness (volume and pressure of the air in the tympanic cavities, tonus of the middle ear muscles, and mechanical immittance of the cochlea). The change in balance between mass and stiffness causes changes in RF. If stiffness of the ear increases, RF is higher than normal. Conversely, if the mass of the ear increases, RF is lower than normal [2]. Because currently available literature is not sufficient, the clinical use of MFT has not become widespread.

Body mass index (BMI) was first described in 1835 by Qutelet. It is a numerical index calculated by dividing body weight (kg) by the square of height (m). Degrees of overweightness are classified according to this index. Age and sex do not affect BMI values [3]. According to the World Health Organization (the 2004 classification), people with BMI of ≥25 are overweight, those with BMI of ≥30 are obese, and those with BMI of <18.50 are underweight [4].

Obesity and overweight have been studied for many years. Obesity affects many systems in an organism and correlates with metabolic diseases such as diabetes mellitus, hypertension, and dyslipidemia. Studies based on imaging methods revealed that BMI correlated with body weight and visceral fat [5, 6]. BMI has also been investigated in people with hearing loss, and although some studies detected a significant association, others obtained significant results only in women or found no association between BMI and hearing [7, 8]. Because an increase in body weight causes an increase in submucosal and visceral fat tissues, in this study of adults with normal-hearing thresholds, BMI values and RF values of the middle ear were compared.

MATERIALS and METHODS

The study included 78 voluntary participants with normal otoscopic examinations and no complaints of hearing loss. The study was approved by the ethics committee of university’s Institutional Review Board (Project no: KA15/313) and was supported by the University Research Fund. All participants provided written informed consent.
The study group comprised 78 volunteers (i.e., 156 ears) with BMI of <18.5 kg/m² (Group 1), BMI of 8.5-24.9 kg/m² (Group 2), and BMI of >25 kg/m² (Group 3). The sample width was calculated in a pre-study statistical evaluation. Individuals with a chronic, immunologic, neurologic, and otologic diseases, a history of drug use were excluded from the study. Patients with had normal hearing (hearing thresholds of ≤15 dB (ANSI 1988)) and a normal tympanic examination (peak impedance value was±50 daPa) and, acoustic reflexes between 500 and 4000 Hz at normal levels were included.

Hearing thresholds were determined by pure tone audiometry in individuals who underwent an otoscopic examination performed by an Ear Nose Throat (ENT) specialist. Pure tone audiometry was evaluated using the Interacoustics AC-40 clinical audiometer (Interacoustics A/S, DK-5610, Assens, Denmark) in a quiet room according to the international acoustic company standard. Air-conduction hearing thresholds were measured using TDH-39 standard headphones. Measurements at frequencies between 125 and 8000 Hz were performed. Bone conduction hearing thresholds were measured using the Radiocure B-71 bone vibrator at frequencies between 250 and 4000 MHz.

Immitansmetric measurements of all participants were performed using the Grason Stadler Tympstar Version 2 electroacoustic immittance meter. First, tympanometric and static admittance values were recorded using a 226-Hz probe tone. The tympanogram was obtained by changing the air pressure from +200 to -400 daPa at a ratio of 200 daPa/s. MFT was then performed in two steps. In the first step, standard tympanometric data such as static admittance, tympanometric peak pressure, and gradient value were assessed by presenting a probe tone at a constant frequency and changing the pressure between +200 and -400 daPa and then a tympanogram was drawn. In the second step, the RF value of the middle ear was assessed by maintaining a constant pressure and successively applying a stimulus within the frequency range of 200 and 2000 Hz to both ears at intervals of 50 Hz; the outputs were then recorded together with other immitansmetric values.

Sex, age, height, weight, BMI, and MFT values of the participants were noted and compared. Data were statistically analyzed using the Statistical Package for Social Sciences for Windows 16.0 software (SPSS Inc.; Chicago, IL, USA) package. Statistical significance was accepted at p values of <0.05.

RESULTS

Each group comprised 26 participants, of which 13 were females and the other 13 were males. The age range of the females was 20-40 years, and that of the males was 18-39 years. Data regarding for sex and means and ranges of age, height, weight, BMI, and RF values for both ears are presented in Table 1. When RF values of the right and left ears were compared, no statistically significant difference was observed (p=0.874). Statistical analysis of the right and left ear RF values according to gender sex summarized in Table 2.

There was no statistically significant difference between RF values of males and those of females (p=0.612; p=1.0). In the analysis of the results, an intergroup analysis of the three BMI groups was performed on the basis of the total number of participants in each group regardless of sex and ear.

The mean RF value was 823.08±86.58 Hz for Group 1, 817.31±94.91 Hz for Group 2, and 771.15±112.61 Hz for Group 3. When RF values were compared between Groups 1 and 2, no significant difference was observed (p=0.747; Table 3). When Groups 1 and 3 were compared, a significant difference was observed (p=0.01; Table 4). When Groups 2 and 3 were compared, a significant difference was observed (p=0.026; Table 5). A negative correlation existed between BMI and RF values (p=0.015).

DISCUSSION

Multifrequency tympanometry is a technique for evaluating the immittance of the middle ear over a wide frequency range. RF values change in different pathologies when either the mass or stiffness of the middle ear system is affected. High-frequency probe tones provide valuable data regarding pathologies such as otosclerosis, osicular chain deformations, middle ear malformations, cholesteatoma, middle ear tumors, and osteogenesis imperfecta, which increase the

<table>
<thead>
<tr>
<th>BMI</th>
<th>n</th>
<th>Mean (Hz)</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>823.08</td>
<td>86.58</td>
<td>0.747</td>
</tr>
<tr>
<td>18.5-24.9 kg/m² (Group 2)</td>
<td>52</td>
<td>817.31</td>
<td>94.91</td>
<td></td>
</tr>
<tr>
<td>&gt;25 kg/m²</td>
<td>52</td>
<td>771.15</td>
<td>112.61</td>
<td></td>
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BMI: body mass index; SD: standard deviation; RF: resonance frequency

<table>
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<td>52</td>
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<td>&gt;25 kg/m²</td>
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<td>771.15</td>
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</table>

BMI: body mass index; SD: standard deviation; RF: resonance frequency

Table 1. Data regarding sex, and means and ranges for age, height, weight, BMI, and RF values for both ears

<table>
<thead>
<tr>
<th>Age (Year)</th>
<th>n</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-40</td>
<td>78</td>
<td>18</td>
<td>40</td>
<td>28.73</td>
<td>6.11</td>
</tr>
</tbody>
</table>

Table 2. Distribution of RF values with regard to sex

<table>
<thead>
<tr>
<th>Gender</th>
<th>RF analysis of the groups with BMI of &lt;18.5 kg/m² and 18.5-24.9 kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>n</td>
</tr>
<tr>
<td>&lt;18.5 kg/m² (group 1)</td>
<td>52</td>
</tr>
<tr>
<td>18.5-24.9 kg/m² (Group 2)</td>
<td>52</td>
</tr>
<tr>
<td>&gt;25 kg/m²</td>
<td>52</td>
</tr>
</tbody>
</table>

BMI: body mass index; SD: standard deviation; RF: resonance frequency

Table 3. RF analysis of the groups with BMI of <18.5 kg/m² and 18.5-24.9 kg/m²

<table>
<thead>
<tr>
<th>BMI</th>
<th>n</th>
<th>Mean (Hz)</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5 kg/m²</td>
<td>52</td>
<td>823.08</td>
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<tr>
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<td>771.15</td>
<td>112.61</td>
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BMI: body mass index; SD: standard deviation; RF: resonance frequency

Table 4. RF analysis of the groups with BMI of <18.5 kg/m² and >25 kg/m²

<table>
<thead>
<tr>
<th>BMI</th>
<th>n</th>
<th>Mean (Hz)</th>
<th>SD</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>18.5-24.9 kg/m²</td>
<td>52</td>
<td>817.31</td>
<td>94.91</td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index; SD: standard deviation; RF: resonance frequency

Table 5. RF analysis of the groups with BMI of 18.5-24.9 kg/m² and >25 kg/m²

[^1]: Sözen et al. The Effect of Body Mass on Resonance Frequency

[^2]: BMI: body mass index; SD: standard deviation; RF: resonance frequency

[^3]: p < 0.05
stiffness of the middle ear system. In addition, studies have shown that RF values can vary according to many factors \cite{1, 6, 9-11}. However, we could not find any data regarding the influence of body weight on RF. This is the first study to demonstrate that RF values are decreased in patients with an increased BMI.

A study of Chinese and Caucasian people revealed that RF values of the Chinese people were higher than those of the Caucasian people \cite{12}. Thus, normalization values for different regions or ethnicities are necessary. In normalization studies of normal-hearing individuals reported in the literature, the mean RF value was between 871 and 1000 Hz \cite{13, 14}. In a study conducted in pregnant women by Dag et al. \cite{15}, significantly lower RF values were obtained in both ears compared with non-pregnant women of the same age range. The author suggested that this difference resulted from an increased endolymphatic fluid, edema in the middle ear mucosa, disruption of the osicular chain, or relaxation of ligaments during pregnancy.

The differences in RF normalization values in different populations reported in the literature are explained based on age and the differences in hereditary characteristics of the middle ear and outer ear structures \cite{2, 6}. However, in studies that compared different age groups, no significant difference was observed with regard to RF values \cite{17, 18}. Furthermore, in a study that investigated RF values according to the body position, although the difference was not significant, there was a difference between the RF value observed in the vertical position and that observed in the Trendelenburg position \cite{19}. In our study, we evaluated all participants in the sitting position, thereby eliminating changes that can occur in different positions. In addition, we obtained a more homogenous group of 18-40-year-old adults by eliminating physical and physiological effects that may occur in children and geriatrics. We did not observe a significant difference in RF values with regard to sex.

In our study, the difference in RF values between sex and right–left ears was not statistically significant. Therefore, all participants were evaluated without considering sex or right–left ear discrimination to investigate how RF values were affected according to BMI. We observed that as BMI values increased, RF values decreased. This finding suggested that alterations in body weight affect ear tissues. BMI is considered to be related to an increased intracranial pressure and body water rates. A previous study showed that an increase in intracranial pressure caused an increase in inner ear pressure. Increased body weights might affect the inner ear mechanics by increasing intracranial pressures. However, this is just a speculation based on current data. One limitation was that the present study was population based and included limited number of subjects. The other limitation was that intracranial pressure and the body water ratio were not measured. Future studies should focus on the mechanisms that influence BMI and middle ear RF.

CONCLUSION
In conclusion, RF values of the middle ear were affected by body weight. However, the mechanism of decreased RF values in obese is not clearly known. Therefore, further studies should focus on the possible causes that lead to abnormal RF.

Ethics Committee Approval: Ethics committee approval was received for this study from Baskent University’s Institutional Review Board (Project No: KA15/313).
Comparison of Cisplatin with Lipoplatin in Terms of Ototoxicity

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OBJECTIVE: Cisplatin (CDDP) is an anti-neoplastic agent that has been used in treatments of both pediatric and adult cancers. It has many side effects, such as ototoxicity, nephrotoxicity, and neurotoxicity. Lipoplatin (LIPO) is a nanomolecule with 110 nm diameter and composed of lipids and CDDP. In this study, we aimed to compare the toxic effects of LIPO with CDDP in the cochlear cells with anti-tumoral doses determined in neuroblastoma cells.

MATERIALS and METHODS: House Ear Institute Organ Corti 1 (HEI-OC1), MYC-N amplified KELLY, and MYC-N non-amplified SH-SY5Y human neuroblastoma cells were used in this study. Firstly, anti-tumoral lethal dose 50 (LD50) of LIPO and CDDP were determined using the WST-1 assay in both neuroblastoma cells. Then anti-tumoral doses of CDDP and LIPO were applied on HEI-OC1 cells for evaluating the toxic effects. The apoptotic cell death was measured using flow cytometric analysis of annexin-V/7-amino-actinomycin (7-AAD) and cell cycle tests.

RESULTS: LIPO or CDDP inhibited cell viability in a dose- and time-dependent manner in both neuroblastoma and HEI-OC1 cells. LD50 values were selected as 20 mM for CDDP and 750 mM for LIPO in neuroblastoma cells. After the 48-hour incubation, KELLY cells treated with 20 mM CDDP and 750 mM LIPO had a 53% viability; SH-SY5Y cells treated 20 mM CDDP and 750 mM LIPO had a 45% and 58% viability, respectively; and HEI-OC1 cells treated with 20 mM CDDP and 750 mM LIPO had a 65% and 82% viability, respectively.

CONCLUSION: LIPO showed less toxic effects in the HEI-OC1 cells compared to CDDP at anti-tumoral doses.

KEYWORDS: Neuroblastoma, Hei-Oc1, lipoplatin, cisplatin, ototoxicity

INTRODUCTION

Neuroblastoma is a common malignancy in childhood and originates from the primitive neural crest cells in the sympathetic nervous system [1-3]. The behavior of neuroblastoma varies according to the presence of the tumor and the status of metastasis [3, 4]. Neuroblastoma constitutes about 8-10% of all childhood cancers and is responsible for 15% of childhood cancer-related deaths [5]. In neuroblastoma, the patient’s age, tumor histology and stage, and cytogenetic and molecular genetic markers are the most important prognostic indicators [6].

Cisplatin (CDDP) is an anti-neoplastic agent that has been used in the treatment of both pediatric and adult cancers [3, 6]. Although CDDP is used in cancer treatments, it has many side effects, such as ototoxicity, nephrotoxicity, and neurotoxicity [6, 7]. Although nephrotoxicity can be controlled and diminished after CDDP treatment, there is no prevention modality against CDDP ototoxicity in clinical practice [8, 9]. CDDP causes damage to the organ of corti in the cochlea, resulting hearing loss, which can lead to social development deficiencies, such as learning problems, in children. Therefore, ototoxicity is an extremely serious problem in childhood [3, 7]. In previous studies, we reported that CDDP shows toxic effects on House Ear Institute-Organ Corti 1 (HEI-OC1) cells [7, 10].

Liposomal platinum is the most promising drug formulation under clinical conditions [11]. Lipoplatin (LIPO) is a liposomally encapsulated form of CDDP [12]. LIPO is a nanomolecule of 110 nm in diameter, which is composed of lipids and CDDP [13]. LIPO has been...
shown to have toxicity lower than CDDP and more drug accumulation in the tumor [12, 14].

In the present study, we aimed to compare the anti-tumor response of LIPO and CDDP in neuroblastoma and the toxic effects in HEI-OC1 cells.

**MATERIALS and METHODS**

This study was in vitro study. This study was performed in accordance with the principles of the Declaration of Helsinki. Informed consent is not required for the current in vitro study.

**Cell Culture and Reagents**

HEI-OC1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% (w/v) L-glutamine. The cells were incubated at 37°C in a 10% CO2 incubator [10]. KELLY (worse prognostic, MYC-N amplified) and SH-SY5Y (good prognostic, non-MYC-N amplified) neuroblastoma cells (DSMZ) were maintained in DMEM (Gibco™) and Rosewell Park Memorial Institute (RPMI)-1640 (Gibco™) containing 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. The cells were incubated at 37°C and 5% CO2 conditions [5, 15, 16]. All reagents were freshly prepared with mediums before all experiments. The cell viability was determined using an automatic cell counter with a trypan blue exclusion test.

**Detection of Cell Proliferation**

The WST-1 assay used to determine cell proliferation is based on the basic principle the conversion of the pink tetrazolium salt (WST-1) to a dark red formazan dye via the succinate-tetrazolium reductase enzyme, which is active only in living cells in the mitochondrial respiratory chain [9]. Mitochondrial dehydrogenase enzyme activity is increased as the number of viable cells increases. As enzyme activity increases, formazan dye production also increases. Therefore, the formazan dye increases in direct proportion to the number of metabolically active cells in a cell culture. Cells were counted and 10⁴ cells/well were seeded with least 6 replicates in a 96-well plate by overnight incubation. The cells were then treated with different concentrations of LIPO and CDDP (10 µM-1000 µM) for 24, 48, and 72 hours. Following the incubation periods, the WST-1 assay was performed by adding 10 µL of WST reagents (Roche) to each well, and HEI-OC1 and KELLY and SH-SY5Y neuroblastoma cells were further incubated at 33°C and 37°C, respectively, for 2 hours. After incubation periods, the absorbances of the cells was measured using the BD Accuri C6 Software (BD Biosciences) [6].

**Detection of Apoptotic Cells**

Apoptotic cell death was determined using annexin-V-fluorescein isothiocyanate (FITC)/7-amino-actinomycin D (7-AAD; BD Biosciences) and Cyclest™ Plus DNA Reagent Kit (BD Biosciences) in all cells. In the normal cells, phosphatidylserine molecules on the cytoplasmic surface of the cell membrane migrate to the outer surface of the cell membrane in the event of cell apoptosis. Annexin-V can be labeled with FITC, a fluorescent substance, which can bind to the phosphatidylserine on the outer surface of the cell [10]. Thus, the apoptotic cells to which FITC is conjugated with annexin-V can be made visible. The principle of the method is based on the extent apoptosis determination using flow cytometry of cells stained with annexin-V-FITC and 7-AAD, a non-vital dye.

**Statistical Analysis**

The Statistical Package for Social Sciences software for Windows, version 15.0, released 2007 (SPSS Inc.; Chicago IL, USA) was used for statistical evaluation of the data. Findings with a p value <0.05 for statistical significance were accepted. Non-parametric Mann-Whitney U test was also used. Each experiment was repeated at least in triplicate for statistical evaluation.

**RESULTS**

**Cell Proliferation Results**

The cell viability of KELLY human MYC-N amplified and SH-SY5Y human MYC-N non-amplified neuroblastoma cells treated with different doses of LIPO (10-1000 µM) and CDDP (10–1000 µM) was determined using the WST-1 assay. LIPO and CDDP inhibited neuroblastoma cell proliferations in a dose- and time-dependent manner (Figure 1, 2).

The doses of CDDP and LIPO applied for KELLY and SHSY cells were also applied for HEI-OC1 cell and cell viability was determined by the WST-1 assay. Also, proliferations of HEI-OC-1 cells were decreased with lower doses of CDDP but a higher dose of LIPO treatments.

In KELLY MYC-N amplified human neuroblastoma cells, the cell viability decreased at a rate of 41% and 75% with the treatment of 1000 µM CDDP and LIPO, respectively, after 24 hours of incubation (p<0.05; Figure 1).

Cisplatin inhibited 50% cell viability at 20 µM doses, while 750 µM LIPO inhibited 50% cell viability in KELLY cells after 48 hours of incubation (p<0.05; Figure 1).

Also, 20 µM CDDP decreased KELLY cell viability at 33%, but 750 µM LIPO inhibited cell viability at 37% after 72 hours of incubation (p<0.05; Figure 1).
CDDP and LIPO significantly decreased the cell viability with 20 and 750 μM concentrations after 24 hours of incubation compared to control cells (p<0.05). CDDP and LIPO significantly decreased the cell viability with 20 and 750 μM concentrations after 72 hours of incubation compared to control cells (p<0.05). CDDP and LIPO significantly decreased the cell viability with 1000 μM concentrations after 48 hours of incubation compared to control cells (p<0.05).

CDDP: cisplatin; LIPO: lipoplatin

In the SH-SYSY MYC-N non-amplified human neuroblastoma cells, cell viability decreased at a rate of 28% and 36% with the treatment of 1000 μM CDDP and LIPO, respectively, after 24 hours of incubation (p<0.05; Figure 2).

Cisplatin inhibited 50% cell viability at 10 μM doses, while LIPO inhibited cell viability at 750 μM in SH-SYSY cells after 48 hours incubation (p<0.05; Figure 2).

Also, 10 μM CDDP decreased SH-SYSY cell viability at 45%, but 750 μM LIPO inhibited cell viability at 42% after 72 hours of incubation (p<0.05; Figure 2).

Cisplatin inhibited 50% cell viability at 50 μM doses, while LIPO inhibited cell viability at 750 μM in SH-SYSY cells after 48 hours of incubation (p<0.05; Figure 3).

Also, 20 μM CDDP decreased SH-SYSY cell viability at 23%, but 500 μM LIPO inhibited cell viability at 58% after 72 hours of incubation (p<0.05; Figure 3).
In this study, the in vitro cytotoxic effects of LIPO and CDDP on HEI-OC1 cells with neuroblastoma cells were also compared. Firstly, HEI-OC1 cells were treated with the same doses of both CDDP and LIPO and the IC50 doses were determined from these experiments.

Moreover, we tested the apoptotic effects of the agents in these cells using the annexin-V and cell cycle analysis.

HEI-OC1 cells treated with 750 µM LIPO for 48 hours had an 82% viability. HEI-OC1 cells treated with 20 µM CDDP for 48 hours had a 65% viability.

Therefore, 20 µM CDDP and 750 µM LIPO concentration applied for 48 hours was designated as the IC50. Treatment with LIPO or CDDP induced a dose-dependent (CDDP, IC50=20 and 50 µM; LIPO IC50=750 and 1000 µM) and time-dependent (48 h) inhibition of cell proliferation.

In the SH-SYSY cells, the cell viability was 57% for LD50 doses of LIPO 750 µM, the lowest dose of CDDP is 50% of deaths from 10 µM achieved, and the dose increases with decreased cell viability. The cell viability was 53% for LD50 doses of LIPO 750 µM and 53% for the 20 µM CDDP in KELLY cells. In the HEI-OC1 cells, 1000 µM LIPO led to 66% cell viability and 50 µM of CDDP decreased the cell viability below 50% (44%).

**Apoptosis Results of the Cells**

We also compared apoptosis level at the IC50 concentration of LIPO and CDDP on KELLY, SH-SYSY, and HEI-OC1 cells. To compare the apoptosis level of LIPO and CDDP, flow cytometry annexin-V/7-AAD and cell cycle assays were used.

LD50 doses of CDDP caused 62.3-77.3% and LIPO caused 38.85-45.6% apoptotic cell death in KELLY cells. In SH-SYSY, LD50 doses of CDDP caused 75.8-86.9% and LIPO induced 25.3-56.3% apoptosis (Figure 4). Cell cycle findings showed similar results in apoptosis (data not shown).

**DISCUSSION**

An ideal chemotherapeutic agent should be effective on cancer cells, and it should not affect normal cells. CDDP is a highly effective chemotherapeutic agent widely used for treating various adult and pediatric cancers; however, it has dose-limiting serious adverse effects, including nephrotoxicity, neurotoxicity, and ototoxicity [1]. It has been shown that CDDP has ototoxic effects in many studies [3,4]. LIPO, a nanomolecule 110 nM diameter, is a liposomal formulation of CDDP and is composed of lipids and CDDP. LIPO was developed to reduce the systemic toxicity of CDDP [12, 13].

Our previous study has shown that CDDP has toxic effects in HEI-OC1 cells [20]. In the present study, we aimed to compare the potential toxic effects of LIPO and CDDP on HEI-OC1 cells. Previous in vitro and in vivo researches have shown that LIPO has less nephrotoxic effects compared to CDDP [22]. Furthermore, chemoradiotherapy with LIPO for advanced gastric cancer treatment demonstrated minor toxicity at phase 1/2 study [23]. Fantini et al. [24] showed that LIPO therapy has lesser renal toxicity than CDDP in lung and breast cancer treatment in clinical studies.

Moreover, LIPO showed anti-tumor effect in CDDP-sensitive and -resistant ovarian cancer cells since apoptosis was induced by caspases 3, 8, and 9 activation; Bax upregulation; and Bcl-2 downregulation [31]. LIPO caused a synergistic effect combination with doxorubicin and the albumin-bound paclitaxel abraxane. Also, LIPO inhibited ovarian xenograft tumor growth with minimum systemic toxicity in that study.

In an animal study to evaluate the renal toxicities of LIPO and CDDP, it has been shown that LIPO induces less structural and functional damage to the kidneys in mice than CDDP [25]. It was observed that intraperitoneal bolus injection of CDDP and LIPO in rat kidneys resulted in less platinum accumulation with LIPO, although CDDP and LIPO reached the same level of platinum.

**CONCLUSION**

In this study, the anti-tumor and apoptotic effect of LIPO was determined in neuroblastoma cells at a higher dose than CDDP and at later time periods. LIPO caused less apoptotic cell death on cochlear cells than CDDP at anti-tumoral doses, suggesting a lower toxicity in cochlear cells compared to CDDP. Further in-vivo comparative studies are needed for understanding the mechanism of ototoxic effects of LIPO versus CDDP [22].

**Ethics Committee Approval:** Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki “Ethical Principles for Medical Research Involving Human Subjects”, (amended in October 2013). Informed Consent: N/A.

**Peer-review:** Externally peer-reviewed.


**Acknowledgements:** The authors would like to thank Prof. F. Kalinec for ensuring HEI-OC1 cells.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study has received no financial support.

**REFERENCES**


There is a paucity of high-level evidence regarding the surgical management of cholesteatoma. This is in part due to a lack of randomized controlled studies in the field of tympanomastoid surgery, as more readily achievable study designs are prone to biases that may not be easily overcome: for example, allocation, observer, and selection bias. Surgeons inevitably select what they consider to be the optimum surgical intervention, based on the varying degrees of factors such as their experience, resource availability, and assessment of individual patient circumstances. Consequently, published appraisals of outcomes from different surgical interventions rely to a large extent on comparison between different surgeons and different institutions. Unfortunately, there is no consensus on the nomenclature of surgical procedures, thus making such comparisons unreliable [1].

In 2017, the European Academy of Otolaryngology and Neurotology (EAONO) and the Japan Otological Society (JOS) produced a “Joint Consensus on Definitions, Classification and Staging of Middle Ear Cholesteatoma”. Via consultation with the international otology community, a common otology dataset could be used to record their surgical outcome. The high level of international consensus on the IOOG categorization of tympanomastoid surgery supports this tool for surgeons to pool their surgical data into a large database for research and comparative audit.
Community, it has become clear that there is great need for common otology data fields that surgeons can collect systematically for comparative audit and research [2]. In 2017, the International Otology Outcome Group (IOOG) was established to address these issues (www.IOOG.net).

The initial IOOG focus was on the development of a new classification of tympanomastoid surgery, as this is performed commonly by most otologists and comprises a wide range of different techniques. The primary aim was to develop a classification system that would encompass all aspects of a surgical technique that are likely to influence the outcome of tympanomastoid surgery. A pre-requisite was to describe interventions using unambiguous nomenclature, to encourage a uniform manner of reporting, and categorize them in a system that would be acceptable to otologists and neuro-otologists internationally by employing a simple, logical, and user-friendly format. This report outlines the principles used to devise the new classification, including use of the Delphi method to ascertain international acceptance [3].

Formulation of the International Consensus on the “Categorization of Tympanomastoid Surgery”

The IOOG Steering Committee recognized that there is a wide variety of surgical techniques employed by surgeons all over the world. Many of these are “hybrid operations,” for example, creating a window in the scutum, front-to-back mastoidectomy, and exclusion of the mastoid cells remnant using cartilage or soft tissue grafts. It was not the intention of the Steering Committee to produce a coding book for surgery. Surgeons are advised to exercise their judgment by placing their surgical procedure into the best-fitting category within the IOOG categorization to aid international comparison, therefore reducing confusion. The IOOG Steering Committee has produced a user guide to aid explanation.

a. Consensus on mastoid operations

To minimize ambiguity, the IOOG Steering Committee used terms that describe what the surgeon does rather than historical terminologies that are open to personal interpretation. For this reason, terms such as “modified radical mastoidectomy,” “radical mastoidectomy,” and “tympanoplasty” were deliberately abandoned.

The IOOG Steering Committee tried to make the description of the surgical procedure compatible with ICD-10 if possible. The label “Mastoidectomy with removal of the bony canal” was used instead of “Canal wall down mastoidectomy”; “Mastoidectomy with canal wall preserved” was used instead of “Canal wall up mastoidectomy.”

The IOOG Steering Committee used the acronym SAMEO to categorize mastoid bone operations, representing the stage of surgery, approach, mastoid bone extirpation, external bony wall repair, and obliteration of the mastoid cavity.

b. Consensus on middle ear operations

The IOOG Steering Committee used the acronym ATO to categorize middle ear operations, with this representing the access to the middle ear, tympanic membrane reconstruction, and ossicular reconstruction. To allow compatibility with ICD-10, the label “repair of tympanic membrane” was used instead of “myringoplasty.” The terms “PORP” and “TORP” were deliberately omitted. The IOOG Steering Committee advises that the SAMEO-ATO scheme should be used as a whole rather than in parts, as the terms “stage of surgery” and “approach” are universally applicable to middle ear operations.

In anticipation of the consensus exercise, the IOOG Steering Committee pre-determined 80% or above as the threshold criteria required to fulfill international consensus. This paper describes the SAMEO-ATO scheme and the methodology on how the IOOG Steering Committee arrived at the final version.

The IOOG international consensus on the categorization of tympanomastoid surgery was based on two cycles of consensus surveys and a field test (Figure 1). The categorization consists of the SAMEO-ATO schemes (Figures 2 and 3) and two schematic drawings that depict the different categories of “Mastoidectomy” and “Ossicular Chain” (Figures 4 and 5). The SAMEO-ATO scheme is complimented by a user guide for explanation and illustration (Appendix 1).

International Consensus Survey Round 1 (Draft 1)

Draft 1 of the categorization document (SAMEO-ATO) was the result of many rounds of discussion and refinement amongst the members of the Steering Committee.

International feedback was sought from international otology societies rather than individuals. Initially, an email in English was sent to the chairpersons of 44 otology societies within the IOOG address book to establish a relationship and to validate the email address contact. There is no an official national otology society for Belgium and the Netherlands. Instead, there is a Dutch–Belgian Otology Group with...
### IOOG categorisation of tympanomastoid surgery: the SAMEO-ATO framework

#### SAMEO for Mastoid surgery

<table>
<thead>
<tr>
<th>S</th>
<th>Stage of surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Primary (first surgery)</td>
</tr>
<tr>
<td>S2p</td>
<td>Planned (2nd look or staged procedure)*</td>
</tr>
<tr>
<td>S3r</td>
<td>Revision (unplanned)*</td>
</tr>
</tbody>
</table>

*2 represents non-primary surgery and not the number of previous surgery

<table>
<thead>
<tr>
<th>A</th>
<th>Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Endoscopic transcanal</td>
</tr>
<tr>
<td>A2</td>
<td>Microscopic transcanal</td>
</tr>
<tr>
<td>A3</td>
<td>Endaural**</td>
</tr>
<tr>
<td>A4</td>
<td>Retroauricular</td>
</tr>
</tbody>
</table>

**A1 and A3 become A3 when an (external/widening) incision is made

<table>
<thead>
<tr>
<th>M</th>
<th>Mastoidectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mx</td>
<td>No mastoidectomy</td>
</tr>
<tr>
<td>M1a</td>
<td>Mastoidectomy with canal wall preserved (cortical mastoidectomy)</td>
</tr>
<tr>
<td>M1b</td>
<td>Mastoidectomy with canal wall preserved (cortical mastoidectomy) + posterior tympanotomy</td>
</tr>
<tr>
<td>M2a</td>
<td>Mastoidectomy with superior scutum removed only (atticotomy)</td>
</tr>
<tr>
<td>M2b</td>
<td>Mastoidectomy with superior scutum and postero-superior canal wall removed (attico-antrostomy)</td>
</tr>
<tr>
<td>M2c</td>
<td>Mastoidectomy with whole canal wall removed (modified radical or radical mastoidectomy)</td>
</tr>
<tr>
<td>M1a+2a</td>
<td>Mastoidectomy with canal wall preserved + atticotomy</td>
</tr>
<tr>
<td>M1b+2a</td>
<td>Mastoidectomy with canal wall preserved + posterior tympanotomy + atticotomy</td>
</tr>
<tr>
<td>M3a</td>
<td>Subtotal Petrosectomy with preservation of otic capsula***</td>
</tr>
<tr>
<td></td>
<td>- exenteration of all mastoid and middle ear pneumatized cells</td>
</tr>
<tr>
<td>M3b</td>
<td>subtotal Petrosectomy with removal of the otic capsula</td>
</tr>
<tr>
<td></td>
<td>- includes labrinthectomy and/or removal of the cochlea</td>
</tr>
</tbody>
</table>

***M3 differs from M3 in that the eardrum is removed intentionally prior to obliteration of cavity and blind pit closure as well as blocking up of the tympanic opening of the Eustachian tube

<table>
<thead>
<tr>
<th>E</th>
<th>External ear canal reconstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex</td>
<td>No external ear canal reconstruction</td>
</tr>
<tr>
<td>E1</td>
<td>Reconstruction with soft materials†</td>
</tr>
<tr>
<td>E2</td>
<td>Reconstruction with rigid materials††</td>
</tr>
</tbody>
</table>

† space behind graft not obliterated

<table>
<thead>
<tr>
<th>O</th>
<th>Obliteration of mastoid cavity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox</td>
<td>No obliteration</td>
</tr>
<tr>
<td>O1</td>
<td>Partial obliteration††</td>
</tr>
<tr>
<td>O2</td>
<td>Total obliteration††</td>
</tr>
</tbody>
</table>

†† Total obliteration is obliteration of the whole mastoid and attic cavities. Partial obliteration spares the attic cavity ± part of mastoid cavity (ie just a reduction of the size of cavity)

---

Figure 2. The IOOG SAMEO scheme of the SAMEO-ATO framework
one Dutch representative and one Belgian representative. For the purpose of this consensus process, the Dutch Otology Group and the Belgian Otology Group were treated as two separate otology societies. Twenty-five out of 44 societies responded. It is possible that the emails sent to others did not reach the intended person due to the use of an outdated email address, or they might have been rejected due to language barriers. The draft SAMEO-ATO scheme, user guide, and the diagrams were sent to the 25 willing chairpersons accompanied by a survey ques-

### IOOG categorisation of tympanomastoid surgery: the SAMEO-ATO framework

#### ATO for Middle ear surgery

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
</table>
| **Ax** | No bone removal from the external ear canal wall  
(flattening of suture line alone is still considered as Ax) |
| **A1** | Widening of the posterior portion of tympanic sulcus  
(including canal curettage or drilling to visualise the ossicular chain or hypotympanum) |
| **A2** | Partial or circumferential widening of the bony canal (canalplasty) |
| **A3** | Total canalplasty with soft tissue grafting of exposed bone

††† The IOOG Categorization does not apply to congenital meatal atresia. A3 differs from A2 in the absence of original canal skin.

<table>
<thead>
<tr>
<th><strong>T</strong></th>
<th>Tympanic membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tx</strong></td>
<td>No tympanic membrane grafting performed</td>
</tr>
<tr>
<td><strong>Tn</strong></td>
<td>Original tympanic membrane preserved</td>
</tr>
<tr>
<td><strong>T1</strong></td>
<td>Supplement to intact tympanic membrane (reinforcement)</td>
</tr>
<tr>
<td><strong>T2</strong></td>
<td>Partial tympanic membrane grafting</td>
</tr>
</tbody>
</table>
| **T3** | Subtotal / total tympanic membrane grafting

†††† Total perforation is defined as complete absence of the tympanic membrane and annulus. Subtotal perforation is the absence of tympanic membrane but the annulus is still preserved.

<table>
<thead>
<tr>
<th><strong>O</strong></th>
<th>Ossicular chain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ox</strong></td>
<td>No reconstruction performed</td>
</tr>
<tr>
<td><strong>On</strong></td>
<td>Intact chain preservation</td>
</tr>
</tbody>
</table>
| **Osi** | Reconstruction between incus and stapes head  
(IS joint repair, bone cement, prosthesis or cartilage) |
| **Osm** | Reconstruction between malleus and stapes head |
| **Ost** | Reconstruction between tympanic membrane and stapes head |
| **Osdr** | Tympanic membrane directly repositioned onto stapes head |
| **Ofi** | Reconstruction between incus and stapes footplate |
| **Ofm** | Reconstruction between malleus and stapes footplate (+ stapes preserved) |
| **Ofst** | Reconstruction between tympanic membrane and stapes footplate (+ stapes preserved) |
| **Oft** | Tympanic membrane directly repositioned onto stapes footplate |
| **Ovi** | Reconstruction between incus and vestibule (including stapedotomy) |
| **Ovm** | Reconstruction between malleus and vestibule (malleo-stapedotomy)  
(+ tissue graft seal over vestibule) |
| **Ovt** | Reconstruction between tympanic membrane and vestibule (+ tissue graft seal over vestibule) |

*Figure 3. The IOOG ATO scheme of the SAMEO-ATO framework*
Figure 4. Schematic illustration of the IOOG categorization of "mastoidectomy".

**IOOG Categorisation of Mastoid Surgery — SAMEO Scheme**

Refer to SAMEO-ATO scheme for detail.

- **Mx**: no mastoidectomy
- **M1a**: canal wall preserved
- **M1b**: + posterior tympanotomy
- **M2a**: only scutum removed
- **M2b**: scutum + postero-superior wall removed
- **M2c**: whole canal wall removed
- **M1a+2a**: combination of M1a and M2a
- **M1b+2a**: combination of M1b and M2a
- **M3a**: subtotal petrosectomy; otic capsule preserved
- **M3b**: subtotal petrosectomy; otic capsule removed
Figure 5. Schematic illustration of the IOOG categorization of "ossicular reconstruction".
Summary of IOOG SAMEO-ATO framework

<table>
<thead>
<tr>
<th>S</th>
<th>Stage of surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Primary (first surgery)</td>
</tr>
<tr>
<td>S2p</td>
<td>Planned (2nd look or staged procedure)</td>
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<tr>
<td>S2r</td>
<td>Revision (unplanned)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A</th>
<th>Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Endoscopic transcanal</td>
</tr>
<tr>
<td>A2</td>
<td>Microscopic transcanal</td>
</tr>
<tr>
<td>A3</td>
<td>Endaural</td>
</tr>
<tr>
<td>A4</td>
<td>Retroauricular</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N</th>
<th>Mastoidectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>No mastoidectomy</td>
</tr>
</tbody>
</table>

| M1a| Canal wall preserved              |
| M1b| + posterior tympanotomy           |

| M2a| Only scutum removed               |
| M2b| Scutum + posterosuperior wall removed |
| M2c| Whole canal wall removed          |

| M1a+2a| Combination of M1a and M2a        |
| M1b+2a| Combination of M1b and M2a        |

| M3b| Subtotal petrosectomy; otic capsule removed |
| M3c| Subtotal petrosectomy; otic capsule preserved |

<table>
<thead>
<tr>
<th>E</th>
<th>External ear canal reconstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex</td>
<td>No external ear canal reconstruction</td>
</tr>
<tr>
<td>E1</td>
<td>Reconstruction with soft materials†</td>
</tr>
<tr>
<td>E2</td>
<td>Reconstruction with rigid materials†</td>
</tr>
</tbody>
</table>

† space behind graft not obliterated

<table>
<thead>
<tr>
<th>O</th>
<th>Obliteration of mastoid cavity</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>Partial obliteration</td>
</tr>
<tr>
<td>O2</td>
<td>Total obliteration</td>
</tr>
</tbody>
</table>

Figure 6. Summary poster of the IOOG SAMEO-ATO scheme for categorization of tympanomastoid surgery
tional Survey Round 1. They were the otology societies from Brazil, Czech Republic, Denmark, Germany, Hong Kong, Hungary, Iran, Italy, Japan, the Netherlands, Romania, Russia, South Korea, Slovenia, Sweden, Switzerland, the United Kingdom, and the United States of America.

During Round 1, all of the chairpersons agreed that there is a need for an international consensus on the categorization of tympanomastoid procedures. Seventeen out of the 18 responders gave approval to the SAMEO-ATO scheme proposed by IOOG, and one disapproved. All 18 responders indicated that they will encourage the use of the SAMEO-ATO scheme for the categorization of tympanomastoid surgery following launch.

The level of approval of each section of the SAMEO-ATO scheme together with selected comments from 18 otology societies in Round 1 are listed in Table 1.

The comments received can be categorized into three main themes:
1. Semantic changes: Based on some of the feedback, the IOOG Steering Committee made changes to the wordings to make them clearer to the users.
2. Mastoidectomy with preservation of the bony canal combined with atticoctomy was not represented: The IOOG Steering Committee added a new category within the revised document (Draft 2) to address this.
3. Level of detail within the SAMEO-ATO scheme: Conflicting comments were received. Some societies wished to have more sub-categories, such as materials of reconstruction, nature of revision surgeries (previous surgery performed in same institution versus elsewhere), the use of active middle ear implants, etc. Other societies advised a more minimalist approach to allow the system to be simpler and more user-friendly. The IOOG decided to take a balanced view and to keep the categorization simple without losing the distinction between important categories. In addition to the Categorization of tympanomastoid surgery, the IOOG intends to produce a common otology dataset for the purpose of comparative audit as a follow-up project. The comments and suggestions received during the consensus exercise will help in the design of this dataset.

International Consensus Survey Round 2 (Draft 2)
Despite a high level of approval of SAMEO-ATO during the first round of the consensus survey (17/18 approval; 1/18 disapproval), the IOOG Steering Committee used the feedback to improve the document. The main change was to insert a category that represents "Mastoidectomy with preservation of the external ear canal in combination with atticoctomy."

The revised SAMEO-ATO scheme (Draft 2) with supporting documents and diagrams was sent again to the chairperson of the 25 otology societies contacted.

| Table 1. The level of approval and comments on Round 1 of consensus survey on the SAMEO-ATO scheme from 18 otology societies (Brazil, Czech Republic, Denmark, Germany, Hong Kong, Hungary, Iran, Italy, Japan, the Netherlands, Romania, Russia, South Korea, Slovenia, Sweden, Switzerland, the United Kingdom, the United States) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Stage Approval | Approach | Mastoidectomy | Ext Ear Canal | Obliteration | Access | Tym Memb | Osciculoplasty |
| Approval | 17 Yes (94%) | 17 Yes (94%) | 17 Yes (94%) | 17 Yes (94%) | 16 Yes (94%) | 17 Yes (94%) | 17 Yes (94%) |
| 1 No | 1 No | 1 No | 1 No | 1 No | 1 abstain 1 No | 1 abstain 1 No | 1 abstain 1 No |
| Comments | | | | | | | | |
| Given Specify if rev alone or with someone else; Identify number of previous op | Considered combination endoscope and incision; Preferred “transcanal to permeatal”; The society that objected insisted that endoscopic approach has no place in mastoidectomy. | Like to specific front-to-back vs back-to-front; Questions if atticotomy is CWU or CWD; Suggest Mod R Mastoid and R Mastoid in separate categories. | Distinguish inflammatory vs congenital atresia; Question if scutumplasty + atticotomy should be CWU or CWD; Suggest fascia or other soft tissue graft group together. | Wish more clarity of A2 and A3 | Wish to see more categories on specific techniques and graft materials; Define partial vs total obtit; Reduce repetition in sub-categories between M and O. | Wish to see more categories on specific techniques and graft materials; Define partial vs total obtit; Reduce repetition in sub-categories between M and O. | Wish a sub-category on active middle ear implant. | Wish a sub-category on active middle ear implant. |
| Ext: external; Tym Memb: tympanic membrane; rev: revision; op: operation; Mastoid: mastoidectomy; CWU: canal wall up; CWD: canal wall down; Mod R Mastoid: modified radical mastoidectomy; R: radical; Oblit: obliteration; VT: ventilation tube |
ogy societies for Round 2 of the consensus survey. The comments received from all the otology societies at Round 1 were anonymized and categorized into themes. They were sent along with Draft 2 to all the chairpersons in compliance with the Delphi method. Again, a consultation period of 2 months was provided to each society.

Out of 25 otology societies, 21 gave their responses in Round 2. The otology societies in Belgium, Canada, and India gave their full approval in Round 2, even though they missed the deadline in Round 1. For Round 2, the responders were the otology societies from Belgium, Brazil, Canada, Czech Republic, Denmark, Germany, Hong Kong, Hungary, India, Iran, Italy, Japan, the Netherlands, Romania, Russia, South Korea, Slovenia, Sweden, Switzerland, the United Kingdom, and the United States of America. There were fewer comments received from the various otology societies in round 2. The comments from the American Otological Society were mixed. They felt that there is no need for a new classification, as historical terminologies are sufficient for reporting. Nevertheless, they were happy with the description and accuracy of the SAMEO-ATO scheme. They were concerned that the complexity of the system may discourage routine use by busy clinicians. As such, they are reluctant to recommend the SAMEO-ATO scheme to be mandatory for the reporting of surgical outcome in their official journal Otology and Neurotology.

The comments received by the IOOG Steering Committee during Round 2 are listed in full rather than grouping them into themes (Table 2). The single society who expressed disapproval during Round 1 provided approval at Round 2. The only disapproval received in Round 2 was from the Dutch otology group regarding “Ear canal wall reconstruction.” They suggested deleting the statement regarding “air behind graft,” as it was considered to lead to confusion. After deliberation, the IOOG Steering Committee decided to retain this statement, but to revise the wording from “endoscopic” Vs “Partial” to “Mastoid” statement about “back-to-front” window in scutum; Vs “rigid.” Suggest deleting the statement about “front-to-back” Vs “microscopic surgery”.

### Table 2. The level of approval and comments on Round 2 of consensus survey from 21 otology societies (Belgium, Brazil, Canada, Czech Republic, Denmark, Germany, Hong Kong, Hungary, India, Iran, Italy, Japan, the Netherlands, Romania, Russia, South Korea, Slovenia, Sweden, Switzerland, the United Kingdom, and the United States)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Approach</th>
<th>Mastoidectomy</th>
<th>Ext Ear Canal</th>
<th>Obliteration</th>
<th>Access</th>
<th>Tym Memb</th>
<th>Ossiculoplasty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approval</td>
<td>21 Yes (100%)</td>
<td>21 Yes (100%)</td>
<td>21 Yes (100%)</td>
<td>20 Yes (95%)</td>
<td>1 No (5%)</td>
<td>21 Yes (100%)</td>
<td>21 Yes (100%)</td>
</tr>
<tr>
<td>Comments</td>
<td>Specify if rev op on own cases Vs elsewhere.</td>
<td>Make distinction between “Mod R Mastoid” Vs “R Mastoid”; Consider category for Intact Bridge Mastoid; Question how to classify “making a window in scutum”; Suggest adding “Partial” to “Ext Ear Canal” in heading; Question how “disease destruction of labyrinth” should be classified.</td>
<td>Some overlap here with O; Suggest adding “/Partial” to “Ext Ear Canal” in heading; Need clarification on terminologies “soft” Vs “rigid.” Suggest deleting the statement about “air behind graft.”</td>
<td>Acronym of E and O are confusing.</td>
<td>More categories based on graft materials; Add a category on completely absent of annulus.</td>
<td>Term “columnellar” should be reserved for TORP only.</td>
<td></td>
</tr>
</tbody>
</table>

Ext: external; Tym Memb: tympanic membrane; rev: revision; op: operation; Mast: mastoidectomy; CWU: canal wall up; CWD: canal wall down; Mod R Mastoid: modified radical mastoidectomy; R: radical; Obl: obliteration; TORP: total ossicular replacement prosthesis.
Presented as O ftm. The IOOG Steering Committee has now introduced labeling on the relevant diagram to clarify this.

The delegates were asked to give a show of hands at the end of the session. Sixty persons indicated approval of the SAMEO-ATO scheme, and none indicated disapproval.

The international consensus on IOOG categorization of tympanomastoid surgery and user guide is presented in Figures 2–5. The IOOG Steering Committee also provides a poster summary of the scheme for users to display in their operating room (Figure 6).

After the launch of the categorization of tympanomastoid surgery, the IOOG Steering Group will organize a multi-center study to measure how well the SAMEO-ATO scheme is holding up.

DISCUSSION

The primary aim of IOOG was to develop an internationally approved categorization of tympanomastoid surgery that would encompass all aspects of surgical technique. This could provide the basis for surgeons to pool their surgical data into a large database for research purpose. There are a number of historical classifications for middle ear and mastoid surgery in the literature. 1 A recent systematic literature review showed that many of these systems are outdated or incomplete; most are not widely accepted and only few correspond with all current surgical techniques. IOOG decided to produce a system based on international consensus.

Much consideration was given to get maximum international representations in the consensus process. The members of the IOOG Steering Committee were from seven countries. The expert group or raters were the representatives or chairpersons of 21 otology societies. The reason for inviting the chairpersons as members of the expert group was because they are the experts and can help in the eventual dissemination of the scheme amongst the members of the societies.

The Delphi technique was chosen for the consensus methodology because it allows time for each chairperson to consult the council members of each society. This could not be done with the Nominal Table 3.

<table>
<thead>
<tr>
<th>Country of Work</th>
<th>Question/Comment</th>
<th>Action Taken by the IOOG Steering Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Zealand</td>
<td>How to categorize TORP in the presence of stapes</td>
<td>General agreement that is Ofm or Oft. Will make this clearer on a diagram</td>
</tr>
<tr>
<td>UK</td>
<td>Can SAMEO-ATO be used retrospectively?</td>
<td>Depends on how detailed the original dataset is. Best is prospectively. The IOOG Steering Group members will try to retrospectively categorize their own surgery and present that in a report</td>
</tr>
<tr>
<td>Spain</td>
<td>Clarification on terminologies under “Approaches”</td>
<td>Reinforce A1 and A2 involve no external incision; Will make &quot;Endoscopic&quot; and &quot;Microscopic&quot; more prominent under the transcanal approach</td>
</tr>
<tr>
<td>USA</td>
<td>Suggest use term &quot;removal&quot; and “preserved” instead of CWU and CWD to make it more compatible with ICD-10</td>
<td>General agreement on the suggestion and changes will be made to the terminology.</td>
</tr>
<tr>
<td>UK</td>
<td>Make a distinction between &quot;subtotal&quot; and &quot;total&quot; TM repair.</td>
<td>Difficulty to define total perforation based on amount of annulus present. Such distinction can be included in the data field set rather be given a separate category</td>
</tr>
<tr>
<td>Belgium</td>
<td>Ovt is a dangerous procedure and should not be performed.</td>
<td>The current scheme is not designed to teach surgeons what to do.</td>
</tr>
<tr>
<td>Canada</td>
<td>Make a separate category under O for total removal of footplate—by accident or by design at surgery.</td>
<td>Most delegates agreed that there is no need to include Ovd as it is extremely rare. Total stapedectomy with soft tissue graft could be included in the data field set rather than given a separate category</td>
</tr>
<tr>
<td>France</td>
<td>Clarify if cartilage plate/sheet over stapes head Ost or Osd.</td>
<td>Ost: If the cartilage strut is inserted between eardrum and stapes, then it is Ost.</td>
</tr>
<tr>
<td>Belgium</td>
<td>Why is middle ear pathology not featured?</td>
<td>The categorization is only for surgical procedure. The IOOG is working on minimal data fields that include all risk factors of chronic ear surgery.</td>
</tr>
<tr>
<td>UK</td>
<td>What about including procedures for complications?</td>
<td>These are rare, and their inclusion will make the system too complicated.</td>
</tr>
</tbody>
</table>

TM: tympanic membrane; Ofm: ossicular reconstruction between the footplate and malleus; Oft: ossicular reconstruction between the footplate and tympanic membrane; Ovd: tympanic membrane directly placed over an open vestibule directly; Ost: ossicular reconstruction between stapes and tympanic membrane; Osd: tympanic membrane directly placed on the stapes head)
Group Technique. The Delphi technique also has the advantage of anonymity and thus avoids dominance by certain groups. The high international approval rating of over 90% on the IOOG SAMEO-ATO scheme supports its use as the international categorization of tympanomastoid surgery.

Peer-review: Externally peer-reviewed.


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3. Humphrey-Murto, Susan MD, MEd, FRCP; Varpio, Lara PhD; Wood, Timothy J, PhD; Gonsalves, Carol MD, MMed; Ufholz, Lee-Anne MLIS; Foth, Thomas RN, PhD. The Use of the Delphi and Other Consensus Group Methods in Medical Education. Acad Med 2016; 91: 11.
Appendix 1. Explanatory notes on the IOOG categorization of tympanomastoid surgery

1. The aim of the SAMEO-ATO categorization is to aid the comparison of surgical outcome from middle ear and mastoid surgery between institutions.

2. SAMEO-ATO is an acronym that outlines the subgroups of mastoid and middle ear surgery that comprise the classification system:
   - **Stage of operation**
   - **Approach**
   - **Mastoidectomy procedure**
   - **External auditory canal reconstruction**
   - **Mastoid Surgery**
   - **Obliteration of mastoid cavity**
   - **Access**
   - **Tympanic membrane (TM) repair**
   - **Middle ear surgery**
   - **Ossicular chain repair**

3. Where possible, terminology in the SAMEO-ATO system is derived from descriptors used in ICD-10-PCS (see www.icd10data.com/ICD10PCS/Codes/0/9). To minimize ambiguity, terms describe what the surgeon does rather than historical nomenclature that may be open to variable interpretation. For this reason, procedures are not grouped by terms such as “atticotomy” (see also below), “modified radical mastoidectomy,” “radical mastoidectomy,” and “tympanoplasty.” Nevertheless, common names such as these are included in brackets to facilitate understanding.

4. The IOOG Steering Committee recognizes that there is great variation in surgical techniques used by surgeons all over the world. The Committee has aimed for a balance in the SAMEO-ATO system between simplicity and over-complexity to provide a usable classification that includes the parameters that distinguish important differences in surgical intervention. Surgeons that perform a procedure that they consider to be significantly different from any of the categories defined by the SAMEO-ATO are encouraged to allocate their procedures to the closest fit. Details of any such differences should be recorded separately to generate data than can be used to stimulate future revisions of the SAMEO-ATO system.

5. Other important parameters that might influence the outcome, including patient-related variables, complications of disease or surgery, and further surgical details such as the nature of previous surgery, grafting materials, concomitant use of ventilation tube and active middle ear implants, are beyond the scope of this surgical classification. The IOOG Steering Committee is developing a common dataset for middle ear surgery to use alongside the SAMEO-ATO system that will include such topics. Of course, additional parameters may be recorded by individual surgeons, but this should not compromise allocation of procedures into the SAMEO-ATO categories.

6. The SAMEO-ATO system has been developed for implementation in prospective data collection. Although it may be possible to apply the system retrospectively, the accuracy of classification will be impaired significantly by the limits of previously collected data fields. Users should report whether the system has been applied prospectively or retrospectively and emphasize potential discrepancies from retrospective use.

7. The IOOG Steering Committee does not advocate any particular surgical procedure or combination of procedures that may be defined by the SAMEO-ATO classification but has simply attempted to derive a comprehensive classification system. An example raised during consensus discussions was that ossiculoplasty type $O_{i}$ might be dangerous so arguably should not be performed, but it remains in the classification for completeness.

### Guidance on implementation of the SAMEO-ATO categorization in tympanomastoid surgery

These notes provide guidance on use of the SAMEO-ATO system. The IOOG advises that surgeries be categorized using all components of the SAMEO-ATO, not limited just to the parts of surgery that are completed (e.g., the absence of any mastoid surgery is categorized as $M_{E_{0}}O_{i}$). “Stage of surgery” and “Approach” are applicable to both middle ear and mastoid operations.

- **Stage of operation**
  - **S$_{1}$** signifies the first surgery for the condition being treated.
    - For example, first operation for cholesteatoma after a previous TM perforation repair
  - **S$_{2}$** signifies any subsequent surgery for that condition, not the number of operations completed
    - For example, third surgery for a recurrence of cholesteatoma after two planned stages of surgery is coded as $S_{2r}$

- **Approach**
  - **A$_{p}$** if an incision is used to access the mastoid, the use of an endoscopic surgery is considered to be an adjunct procedure
    - For example, post-auricular incision used for endoscopic access to the mastoid is $A_{p}$ even if no microscope is used
  - **A$_{e}$** if an external incision is made for harvest of a graft but not used for access to mastoid or middle ear, this is classified as $A_{e}$
  - **A$_{i}$** The term “permeatal approach” is considered to be synonymous with “transcanal approach.”

### Mastoid Surgery

- **Mastoidectomy procedure**
  - **M$_{1}$** An M1 procedure is a mastoidectomy leaving the canal wall intact (preserved), and an M2 procedure is a mastoidectomy with the removal of the canal wall partly or completely.
  - **M$_{2a}$** As part of the canal wall is removed with the removal of the scutum (atticotomy), this procedure is categorized according to the SAMEO-ATO with other “canal wall down” surgeries. However, many surgeons incorporate an atticotomy and scutum reconstruction with a cortical mastoidectomy in what is considered to be “canal wall up” surgery. This hybrid procedure is defined in the SAMEO-ATO by combining the mastoid codes for cortical mastoid-
ectomy and scutum removal (e.g., M1a,2a or M1b,2a if posterior tympanostomy is included).

iii. Partial scutum removal to create only a window at the scutum (for access to the epitympanum) while preserving its inferior border is also to be classified as M1a,2a.

iv. M2a differs from M2c in that the cavity is closed off completely with an ear canal closure (removal of all ear canal skin and TM), as well as blocking up of the tympanic opening of the Eustachian tube.

- **External auditory canal reconstruction**
  i. Different materials may be used to reconstruct the scutum and/or bony canal wall with the intention of leaving a ventilated attic and mastoid under the graft.

- **Obliteration of mastoid cavity**
  i. O1 means that an empty air space is present behind the ear canal or in the cavity.
  ii. Partial obliteration spares the attic cavity plus part of the mastoid cavity (i.e., just a reduction of the cavity size). A total obliteration is a complete obliteration of the whole mastoid and the attic cavity.
  iii. Obliteration of the attic without obliteration of the mastoid is considered to be O2.
  iv. The type of obliteration material can be added to with small letters in own database and should be reported when presenting a series.

**Middle Ear Surgery**

- **Access**
  i. The distinction between A1 and A2 is that in A2, there is an absence of the meatal skin to line the ear canal, for example, during surgery for medial canal fibrosis.
  ii. This Access category has not been developed for the congenital meatal atresia surgery.

- **TM repair**
  i. Tn TM normal, no need for surgery
  ii. T1 TM not normal, but not repaired

- e.g., atelectatic TM elevated but not reconstructed; perforation present, but surgeon chose not to repair; or previous cartilage tympanoplasty
  iii. T3 Total perforation is defined as complete absence or removal of the TM and annulus. Subtotal perforation is the absence of TM, but the annulus is still preserved.

- **Ossicular chain repair**
  i. O1 Normal ossicular chain. Ossicular repair not needed
  ii. O2 Ossicles not normal, but no ossiculoplasty performed

- **Ossiculoplasty diagrams**
  i. It should be noted that the ossiculoplasty diagrams are conceptual and not a surgical illustration of technique. The stapes superstructure is shown in faded outline (O1m and O1f) to indicate that a reconstruction can be performed whether superstructure is absent or present.
  ii. Categories of ossiculoplasty are defined by the furthest points of contact of the graft or prosthesis between these anatomical structures:
    - m malleus handle
    - t TM
    - i incus
    - s superstructure of stapes
    - f footplate of stapes
    - v vestibule (no distinction is made between footplate perforation, footplate removal, or placement of a soft tissue graft, although these differences may be recorded for presenting/reporting on the dataset)
    - d direct coupling of TM without a graft or prosthesis
      e.g., Osd is myringostapediopexy
  iii. Regarding cartilage tympanoplasty with absent incus but intact stapes:
    - O1d Flat cartilage graft used in TM reconstruction in contact with stapes (cartilage myringostapediopexy)
    - O1s Shaped piece of cartilage placed as a bridge between stapes and TM
  iv. Placement of a silastic band around the stapes superstructure and the shaft of a total ossicular replacement prosthesis between footplate and TM is included within O1n and O1m.

Yung et al. Categorization of Tympanomastoid Surgery
Appendix 2. Survey Questionnaire
The International Otology Outcome Group wishes to get an international consensus on the categorization of tympanomastoid surgery. This is the first step toward starting an international audit of surgical outcomes in chronic otitis media. It will help us if you can give your approval/disapproval of the SAMEO-ATO system designed by members of the Steering Group. Please answer yes or no on the following questions. If you disagree, please give your reasons and suggestions for improvement. Please put yes or no and any comment for each question. **We like to have one response from each otology association.**

1. Do you think there is a need for an international consensus on the definition and categorization of tympanomastoid surgery?
   - Yes / No ……………………………………….
   - Comment…………………………………….

2. Do you approve the description of the mastoidectomy procedure using the system of SAMEO (stage of surgery, approach, mastoidectomy, external ear canal reconstruction, and obliteration)?
   - Yes / No ……………………………………….
   - Comment…………………………………….

3. Do you approve the description of the tympanoplasty procedure using the system of ATO (access to middle ear, tympanic membrane reconstruction, ossicular chain reconstruction)?
   - Yes / No ……………………………………….
   - Comment…………………………………….

4. Do you approve the description under S?
   - Yes / No ……………………………………….
   - Comment…………………………………….

5. Do you approve the description under A (Approach in Mastoidectomy)?
   - Yes / No ……………………………………….
   - Comment…………………………………….

6. Do you approve the description under M?
   - Yes / No ……………………………………….
   - Comment…………………………………….

7. Do you approve the description under E?
   - Yes / No ……………………………………….
   - Comment…………………………………….

8. Do you approve the description under O (Obliteration)?
   - Yes / No ……………………………………….
   - Comment…………………………………….

9. Do you approve the description under A (Access to Middle Ear)?
   - Yes / No ……………………………………….
   - Comment…………………………………….

10. Do you approve the description under T?
    - Yes / No ……………………………………….
    - Comment…………………………………….

11. Do you approve the description under O (Ossicular Chain Reconstruction)?
    - Yes / No ……………………………………….
    - Comment…………………………………….

12. Would you recommend the members of your society to use this in recording their operations?
    - Yes / No ……………………………………….
    - Comment…………………………………….
Classifications of Mastoid and Middle Ear Surgery: A Scoping Review

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Department of Otolaryngology, East Suffolk and North Essex NHS Foundation Trust, Heath Road, Suffolk, United Kingdom (MY)

OBJECTIVES: The aim of this scoping review was to evaluate existing classifications of surgical procedures of the middle ear and mastoid and find a suitable classification that could serve as an international standard.

MATERIALS and METHODS: Scoping review with a systematic literature search using reference tracking and a syntax including all surgical procedures in mastoid and middle ear surgery and their synonyms. Studies were selected based on inclusion and exclusion criteria.

RESULTS: Eleven reported classifications were included; six of which focused on middle ear surgery, two on mastoid surgery, and three on both. However, none of the classifications included all current surgical procedures of mastoid and middle ear surgery.

CONCLUSION: Many classifications have been proposed for innumerable surgical techniques in middle ear and mastoid surgery. Some are outdated, some are incomplete, most are not widely accepted, and only few correspond with all current surgical techniques.

KEYWORDS: Classification, registry, ear surgery, review, middle ear, mastoid

INTRODUCTION

There are a great variety of surgical techniques for diseases of the middle ear and mastoid bone. In order to have meaningful comparison of surgical outcomes, it is important that surgeons use standardized terminologies to describe their operations.

To date, there is no consensus regarding a standardized categorization of tympanomastoid surgery; however, there are a number of classifications based on historical terminologies. This leads to difficulties in making meaningful comparisons or drawing evidence-based conclusions. An internationally approved classification will make it possible to compare and combine surgical series more effectively. This could help in the development of high level evidence and in the creation of evidence-based guidelines.

Over the years, many classifications and categorizations of the middle ear and mastoid surgery have been proposed. For instance, the one presented by Wullstein in 1956 on tympanoplasty is well known and still used today [1]. Conversely, some recent papers do not use the Wullstein classification and present data which is categorized differently or not categorized at all. This non-standardized fashion of presenting data will impede the conduction of meta-analysis or creation of “big data” to overcome the problem of surgical series and to have a higher level of evidence. The International Otology Outcome Group aims to create a better basis for international outcome reporting which will facilitate international collaboration that can lead to the development of high level evidence in otologic surgery. A starting point is to find a standardized way to describe tympanomastoid procedures. One way to achieve this is by grouping the surgical procedures into distinguishable categories. A good classification system should be user-friendly, accepted by the international communities, and include all common surgical techniques. Ideally, it is flexible enough to be used as future proof because tympanomastoid surgery continues to evolve. Some notable examples of recent development are mastoid obliteration and endoscopic ear surgery. This scoping review was set out to present an overview of the available classifications on tympanomastoid surgery and to evaluate their current usability. The aim was to find a suitable classification that could serve as an international standard. This scoping review comprises a systematic literature search, a thorough coverage of available classifica-
tions, and an assessment on its potential to serve as an international standard [2].

METHODS
The approach to this scoping review was conducted according to the Arksey and O’Malley framework [2]. This type of scoping review has a framework that facilitates a comprehensive literature search, a thorough coverage of available literature, and is suited to identify research gaps.

Search Strategy
The search strategy involved searching via electronic databases and reference lists. The electronic database search included Cochrane, PubMed, and Embase and was performed on the March 25, 2018. Keywords used for the search combined various synonyms and types of terminology used for classification and tympanomastoid surgery. Specific types of middle ear and mastoid surgeries were included to maximize the likelihood of capturing all eligible studies. The detailed search syntax can be found in appendix 1.

Selection Criteria
Titles and abstracts were screened; studies that seemed relevant to the research question were read in full text (books and journal papers). When relevance was unclear from the abstract, the study was completely read. Criteria were devised post-hoc because the relevance of the selected studies could be determined more effectively on increasing familiarity with the literature [2]. To be considered for inclusion, studies had to introduce a new or modified classification of middle ear and/or mastoid surgery. Studies were selected for inclusion after approval of all authors. Exclusion criteria included studies not written in English [16-22] and three studies were excluded due to a low number (<5) of citations [23-25]. Six studies met our criteria and five studies [26-30] were added through reference tracking, resulting in a total of 11 studies that were included in this scoping review [1, 26-35].

Study Characteristics
Table 1 summarizes the characteristics of the included studies. Three of the eleven studies included both a middle ear and mastoid surgery classification. The total number of middle ear surgery classifications was nine, of which six solely focused on the middle ear. Five of the middle ear classifications were a modification of the Wullstein classification [1]. One classification consisted solely of ossiculoplasty and another exclusively focused on cartilage tympanoplasty [31, 32]. Mastoid surgery was included in five classifications, which did not include any revisions or modifications of earlier classifications. The oldest classification included was published in 1956 and the newest in 2008. Eight classifications were made by a single author, one was formed by a single center and two by national otology societies [30, 33]. However, no classifications were based on international otologic societies or consensus.

Classification overview
The Wullstein classification [1] describes the degree of damage found in the middle ear and the method of reconstruction. Five modification proposals for this classification have been included in this scoping review. In 1971, Farrior [26] introduced a classification, which preserves the Wullstein classification, and included a scheme for describing some ossicle modifications. Bellucci [27] proposed a dual classification comprising a nomenclature of the stability of the ear against infection and the original Wullstein classification. Pratt [34] modified the Wullstein classification by introducing a sixth type of tympanoplasty comprising the removal of the footplate of the stapes and describing how the mastoid was managed. The Nadol and Schuknecht [29] modification included the removal

Figure 1. Flowchart with the performed selection process. The literature search was conducted on March 25, 2018.
<table>
<thead>
<tr>
<th>Source</th>
<th>Type of classification</th>
<th>Original or modified classification</th>
<th>Consensus based</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wullstein classification:</strong>&lt;br&gt; Type I: Middle ear practically restored to normal; tympanic membrane and middle ear intact.&lt;br&gt;Type II: Middle ear of approximately normal size is tried to preserve in spite of slight defects of the ossicles.&lt;br&gt;Type III: Large defects of the malleus and incus warrant the removal of the ossicular chain and of the epitympanum. The tympanic membrane must be directly connected with the head of the stapes.&lt;br&gt;Type IV: The stapedial footplate is movable, but the stapes is missing. The tympanoplasty reconstructs a middle ear comprising only of the tube and the hypotympanum with sound protection for the round window.&lt;br&gt;Type V: The stapedial footplate is fixed and a fenestra novalis is necessary.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Classification of the common operations performed in surgery for chronic ear infection:</strong>&lt;br&gt;A: Radical or modified radical mastoidectomy&lt;br&gt;B: Mastoid obliteration operation&lt;br&gt;C: Myringoplasty</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farrior [26] 1971</td>
<td>Tympanoplasty</td>
<td>Modified Wullstein classification</td>
<td>Single author</td>
<td>19</td>
</tr>
<tr>
<td><strong>Classification of tympanoplasty type III and IV (modification of Wullstein):</strong>&lt;br&gt;Type IV: No columella&lt;br&gt;Type IV: Incus graft&lt;br&gt;Type IV: Malleus graft&lt;br&gt;Type IV: Bone graft&lt;br&gt;Type IV: Cartilage graft with stainless steel&lt;br&gt;Type IV: Homograft drum with malleus, incus and stapes</td>
<td>Type III: Drums on stapes&lt;br&gt;Type III: Incus graft&lt;br&gt;Type III: Malleus graft&lt;br&gt;Type III: Bone graft&lt;br&gt;Type III: Malleus graft&lt;br&gt;Type III: Malleus, incus and stapes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bellucci [27] 1973</td>
<td>Tympanoplasty</td>
<td>Modified Wullstein classification</td>
<td>Single author</td>
<td>87</td>
</tr>
<tr>
<td>Pratt [34] 1974</td>
<td>Tympanoplasty Mastoidectomy</td>
<td>Modified Wullstein classification</td>
<td>Single author</td>
<td>5</td>
</tr>
<tr>
<td><strong>Modified Wullstein and Farrior classification by adding a sixth type of tympanoplasty and describing how the mastoid was managed:</strong>&lt;br&gt;Type VI: The removal of the stapes footplate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Management of the mastoid, UDT classification:</strong>&lt;br&gt;Surgical technique&lt;br&gt;U: Canal wall up&lt;br&gt;D: Canal wall down</td>
<td>Surgical region&lt;br&gt;II: Attic&lt;br&gt;II: Antrum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tos [28] 1993</td>
<td>Mastoidectomy</td>
<td>Original</td>
<td>Single author</td>
<td>233</td>
</tr>
<tr>
<td><strong>Tos subclassification:</strong>&lt;br&gt;Canal wall up&lt;br&gt;1. Simple/ cortical / complete / Schwartz's mastoidectomy&lt;br&gt;2. Classic intact canal wall mastoidectomy / combined approach tympanoplasty (CAT)</td>
<td>Canal wall down&lt;br&gt;Atticotomy&lt;br&gt;Radical mastoidectomy&lt;br&gt;Modified radical mastoidectomy / Bondy's procedure</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
of types IV and V and the subdivision of type III into three categories: stapes columella, minor columella, and major columella. In 2007, Kim [33] introduced the most recent modification of Wullstein by adding type 0, representing tympanic membrane reconstruction with no hearing gain, and by removing type V. This classification also comprises mastoidectomy, extraneous procedures, and concurrent procedures, such as obliteration. Lierle [30] classified the most common surgeries performed for chronic ear infections into five types, including both tympanoplasty and mastoid surgery. Marres [35] introduced a mastoidectomy classification comprising the performed technique (canal wall up or canal wall down) and a division of the surgical region of the attic and mastoid into four different areas. In 1993, Tos [32] proposed a mastoidectomy classification comprising the surgical technique (canal wall up or canal wall down) and further subdivisions of the technique. McGee [31] proposed a dual classification of ossicular reconstruction comprising a description of the pathology (subdivided in five types) and the associated surgery. Lastly, Tos [32] proposed a cartilage tympanoplasty classification by categorizing the 23 known cartilage tympanoplasty methods into six main groups.

DISCUSSION
This scoping review was intended to present an overview of the existing classifications of middle ear and mastoid surgery. The classifi-
cations were evaluated with regard to their applicability, and the aim was to find a suitable classification that could serve as an international standard.

The literature search identified 11 classifications of middle ear and mastoid surgery published in journals and books from 1956 to 2008. Six classifications focused on middle ear surgery, two on mastoid surgery, and three on both (Table 1).

An internationally approved classification should be well accepted, unambiguous, and encompass all common and current surgical techniques.

The number of citations of each classification roughly reveals the number of studies that used this classification to report their data, to give an idea of international “acceptance.” Six out of eleven classifications were cited less than 20 times and only two were based on national consensus [26, 30]. None were based on international consensus. The middle ear surgery classification of Wullstein and the mastoid classification of Tos are the most cited and still used today [1, 28]. However, these two classifications could not be directly translated into a broadly accepted international classification. The Wullstein classification describes five methods to reconstruct the middle ear and has formed the basis of modification classifications promoted by others [26, 27, 30, 33, 34]. The classification includes many good points but does not accommodate new surgical options, such as a prosthesis from the footplate to the tympanic membrane in the presence of the stapes superstructure [26]. Even in the newer Wullstein modifications [29, 33], some middle ear reconstructions were not included, such as prosthesis from incus/malleus to vestibule as in stapes surgery. Another popular classification is by Tos on mastoid procedures [28]. Tos deliberately maintained the historical terminologies of simple mastoidectomy (cortical, complete, Schwartzte) for translational purpose. It also concludes five categories for a canal wall down procedure (refer to Table 1), one of the categories comprises two techniques (modified radical mastoidectomy and Bondy’s procedure). However, it is unclear if a (modified) radical mastoidectomy truly differs from a retrograde mastoidectomy, other than the approach to surgery.

Many of the classifications were incomplete in their categorization of surgical techniques and miss out some of the current surgical approaches. For instance, mastoid obliteration was described in only two classifications, [30, 31] and techniques that are often used during an endoscopic approach (atticotomy and atticoantrotomy) are missing in some mastoid classifications [26, 30]. In addition, some classifications only focused on specific procedures, such as the Tos’s classification on cartilage tympanoplasty [30]. The Korean Otologic Society has presented a classification, which is close to a comprehensive overview of existing terminologies, yet it has limited structure [33]. For example, a mastoidectomy is labeled either “canal wall up” or “canal wall down,” but missed out on other additional or optional procedures.

Many classifications identified in this study used historical or ambiguous terminologies that are open to personal interpretation and do not correspond with the current ICD-10 nomenclature. “Tympanoplasty” is used in Wullstein for myringoplasty as well as for ossicular chain reconstruction, which leads to confusion among other classifications in which “tympanoplasty” is used only for ossicular chain procedures and “myringoplasty” is separately mentioned [1, 30]. Bellucci used the terms minor- or severe ossicular defect, with further definition missing [27]. Other terms were outdated, such as “cavum minor,” created by a medialized tympanic membrane [27] and “fenestra vernex” [1]. Some terms are new, such as “mastoidotomy” in the same classification as “mastoidectomy” and “atticotomy” [33].

In the classification suggested by Pratt [34], there are five types of mastoidectomy, but strangely enough, one of them is not a mastoidectomy but a type of reconstruction (reconstruction of the posterior wall). Classifying both procedures with the same abbreviation (M) seems illogical as both techniques could be performed in the same procedure.

With regard to the above limitations, the authors could not identify an existing classification that could serve as an international standard. The international otologic society needs to start an international consensus project on the classification of middle ear and mastoid surgery. This could aid international collaboration and greatly improve evidence-based clinical research to create more “big” data to improve surgical outcomes, and facilitate international consensus and evidence for guidelines.

CONCLUSION
Many classifications have been proposed for innumerable surgical techniques in middle ear and mastoid surgery. Some are ambiguous, some are incomplete, most are not widely accepted and only few correspond with most of the current surgical techniques. There is no single classification that could meet what is currently needed to start an international outcome comparison for middle ear and mastoid surgery.

Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES
**Appendix 1.** Search was performed on the 25th of March.

<table>
<thead>
<tr>
<th>Database</th>
<th>Search syntax</th>
<th>Hits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cochrane</td>
<td>&quot;classification&quot;:ti,ab,kw or &quot;definition&quot;:ti,ab,kw or &quot;terminology&quot;:ti,ab,kw or &quot;nomenclature&quot;:ti,ab,kw or &quot;categorization&quot;:ti,ab,kw or &quot;grade&quot;:ti,ab,kw or &quot;grading&quot; or &quot;theory&quot; AND &quot;mastoid&quot;:ti,ab,kw or &quot;typanic membrane&quot;:ti,ab,kw or &quot;ear&quot;:ti,ab,kw or &quot;tympanoplasty&quot;:ti,ab,kw or &quot;myringoplasty&quot;:ti,ab,kw or &quot;stapes&quot;:ti,ab,kw or &quot;typanic&quot;:ti,ab,kw or &quot;otology&quot;:ti,ab,kw or &quot;otologic&quot;:ti,ab,kw or &quot;tympanomastoid&quot;:ti,ab,kw or &quot;mastoidectomy&quot;:ti,ab,kw or &quot;stapedotomy&quot;:ti,ab,kw or &quot;stapedectomy&quot;:ti,ab,kw or &quot;ossiculoplasty&quot;:ti,ab,kw or &quot;atticotomy&quot;:ti,ab,kw or &quot;petrosectomy&quot;:ti,ab,kw or &quot;tympanotomy&quot;:ti,ab,kw AND &quot;surgery&quot;:ti,ab,kw or &quot;procedure&quot;:ti,ab,kw or &quot;surgical&quot;:ti,ab,kw</td>
<td>118</td>
</tr>
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<td>PubMed</td>
<td>(((((((((((classification[MeSH Terms]) OR definition[Title/Abstract]) OR terminology[Title/Abstract]) OR nomenclature[Title/Abstract]) OR categorization[Title/Abstract]) OR grade[Title/Abstract]) OR grading[Title/Abstract]) OR theory[Title/Abstract]) OR classification[Title/Abstract]) AND (((((((((((((((((((((mastoid[MeSH Terms]) OR tympanic membrane[MeSH Terms]) OR ear[MeSH Terms]) OR tympanoplasty[MeSH Terms]) OR myringoplasty[MeSH Terms]) OR stapes[MeSH Terms]) OR mastoid[Title/Abstract]) OR tympanic[Title/Abstract]) OR ear[Title/Abstract]) OR otology[Title/Abstract]) OR otologic[Title/Abstract]) OR tympanomastoid[Title/Abstract]) OR myringoplasty[Title/Abstract]) OR tympanoplasty[Title/Abstract]) OR stapedotomy[Title/Abstract]) OR stapedectomy[Title/Abstract]) OR ossiculoplasty[Title/Abstract]) OR mastoidectomy[Title/Abstract]) OR atticotomy[Title/Abstract]) OR petrosectomy[Title/Abstract]) OR tympanotomy[Title/Abstract]) AND (((Surgery[Title/Abstract]) OR procedure[Title/Abstract]) OR surgical[Title/Abstract])</td>
<td>1269</td>
</tr>
<tr>
<td>EMBASE</td>
<td>(((((classification/exp OR 'definition':ti,ab OR 'terminology':ti,ab OR 'nomenclature':ti,ab OR 'categorization':ti,ab OR 'grade':ti,ab OR 'grading':ti,ab OR 'theory'/exp OR 'theory') AND ('surgery':ti,ab OR 'procedure':ti,ab OR 'surgical':ti,ab) AND ('mastoid'/exp OR 'eardrum'/exp OR 'ear'/exp OR 'tympanoplasty'/exp OR 'myringoplasty'/exp OR 'stapes'/exp OR 'stapod':ti,ab OR 'tympanic':ti,ab OR 'otology':ti,ab OR 'otologic':ti,ab OR 'tympanomastoid':ti,ab OR 'myringoplasty':ti,ab OR 'tympanoplasty':ti,ab OR 'stapedotomy':ti,ab OR 'stapedectomy':ti,ab OR 'ossiculoplasty':ti,ab OR 'mastoidectomy':ti,ab OR 'atticotomy':ti,ab OR 'petrosectomy':ti,ab OR 'tympanotomy':ti,ab))</td>
<td>2573</td>
</tr>
</tbody>
</table>
INTRODUCTION

Delayed facial palsy (DFP) is an uncommon complication after middle ear surgery; it occurs after >72 hours of an uneventful ear surgery [1]. Shea described this phenomenon as “five and a half day syndrome,” meaning that all his personal observations shared the same time lag from surgery. [2] DFP after stapedectomy is a very rare event (0.22%) [3]. Its incidence in the literature ranges between 0.07% and 1.4%. [4-6] While immediate facial palsy is easily explained by the use of local anesthetics (if transient) or by intraoperative severe surgical trauma (if permanent), many hypotheses have been proposed about the late facial nerve dysfunctions, including reactivation of a quiescent virus colonizing the nerve ganglion. According to Shea et al. [4] the most probable etiopathogenetic mechanism for DFP is the activation of a latent herpesvirus in the geniculate ganglion, induced by mechanical stimulation or reactive inflammation of the facial nerve during middle ear surgery. This hypothesis has been supported by many other observations after otological surgical procedures [7-18] and preventive antiviral therapy has been proposed in patients with positive history to varicella zoster virus and herpes simplex virus 1 and 2. However, serological search of a viral etiology is often inconclusive, not always identifying which virus (HSV type 1, 2, or VZV) is involved; the proposed use of preventive therapy with antivirals in all stapedectomy patients is still debated and often not applied in clinical practice [4,7].

The purpose of this work is to review the relevant literature on DFP after stapedectomy, aiming at properly assessing the viral etiology and at identifying factors that might influence the prognosis or the recovery time or the choice of therapies, and describe a very unusual case of DFP characterized by VZV reactivation showed by the eruption of typical RH lesions.

MATERIALS and METHODS

A PubMed search encompassing all publications over the last 40 years was initially performed searching for “facial palsy OR paralysis OR paresis” AND “stapedotomy OR stapedectomy OR stapes surgery” using Boolean combinations; Further, the terms “delayed OR late” were added to the search. The date of last search was 18th March 2018. After screening all the articles abstracts, full-text works pertinent
to DFP after stapedectomy were retrieved and analytically reviewed. Another PubMed search was conducted by searching for the “revision” stapes surgery utilizing the same Boolean combinations: the resulting abstracts were screened for relevance to account for all published cases of DFP. Chronology of onset and remission of the postoperative facial palsy, operated side, age and sex of patients, predisposing factors, surgical technique and tools, intraoperative observations, serological assays, pharmacological therapies, and facial outcomes were looked for in each article and tabulated on a Microsoft Excel spreadsheet. The heterogeneity of case-reports data set and case-series data set did not allow performing a pooled extraction analysis or meta-analysis regarding the effects of surgical procedures and DFP postoperative treatment. No language restrictions were applied and systematic review of the literature has been summarized. In addition to the literature search, a case of DFP with unique features of VZV infection in an adult who had undergone stapedectomy is described and the findings are compared with similar reports in scientific publications. The House–Brackmann (H-B) facial nerve grading system (1985) was used to evaluate the facial function in patients with DFP after stapedectomy and at the end of the recovery period.

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on human experimentation (IRB 2016) and with the Helsinki Declaration of 1975, as revised in 2008. The patient has given written consent for publication.

Statistical Analysis
Descriptive statistical analysis was performed using Statistical Packages for the Social Sciences (SPSS) software for Mac version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS
Till date, 48 cases of DFP after stapes surgery have been described in the literature, including the present case; 33 of them occurred after stapedectomy and 14 after laser stapedoplasty. DFP were evenly distributed across age and sex and usually develops rapidly into complete palsy after few hours of onset. (Table 1) In all reported cases, the stapes surgeries were uneventful. In the literature, the onset time of DFP varies from 4 to 20 days after surgery (8.5±3.5 days). Interestingly, no specific macroscopic or otomicroscopic signs are usually observed neither reported in cases series; conversely, typical RH is characterized by the typical appearance of VZV vesicles in the conchal area and at the entrance of the external auditory meatus, facial palsy, hearing loss, and intense pain. The association of increased anti-HSV 1-2 and VZV IgM with typical RH syndrome with ear pain and presence of vesicles at the entrance of the ear canal represent the most reliable indicators of VZV reactivation. However, after uncomplicated stapedectomy reported in the literature, typical RH was also absent in patients whose serologic tests proved positive. The recovery time after DFP onset ranges from 2 to 270 days (mean 45±43 days) with H-I final H-B grade in all the reported cases, regardless of the therapies.

A retrospective review of all subsequent operated patients during the last decade has been currently obtained for the purpose of this study. In our casistics, 1253 stapedectomies were personally performed by the same operator between January 1992 and May 2016, using the same conventional technique of footplate fenestration (0.4 to 0.6 mm calibrated hole) with a microdrill without connective tissue interposition. In the entire personal series, DFP occurred only in one patient, accounting for an incidence of 0.08%.

This patient was a 51-year-old female, admitted for bilateral otosclerosis, who underwent right stapedectomy under local anesthesia and interposition of a standard platinum-fluoroplastic piston (Audio Technologies, Piacenza, Italy) measuring 0.6 × 4.5 mm through a 0.7 mm platinnotomy hole obtained using a microdrill. Preoperative pure tone audiometry showed bilateral up-sloping conductive hearing loss with a pure tone average of 58.7 dB HL in the right ear and 48.6 in the left; the mean air-bone gap (ABG) was 40 dB HL and 35.2 dB HL respectively. The middle ear anatomy was normal; in particular, the Fallopian canal was intact. Chorda tympani nerve was not stretched neither manipulated; no intraoperative complications were encountered.

The postoperative course was uneventful, except for slight dizziness induced by head movements during the first 12 hours. She was discharged on the 1st postoperative day with prescription of 400 mg oral cefixime q.i.d. and local instillation ear drops (0.3% ofloxacin solution b.i.d.) for 5 days.

On the 12th postoperative day, the patient complained of intense pain in the right (operated) ear, which was only partially relieved by anti-inflammatory drugs. On the 15th day, she noticed facial paresis on the operated side. The paresis progressed to complete palsy (H-B grade VI) within 24 hours. Painful edema of the soft tissues of the outer ear canal was observed; the retroauricular skin was hyperemic.

The patient had a positive history for chickenpox during childhood; she reported suffering of herpes labialis, once adult, due to sporadic reactivation of HSV type 1 and 2, which has been serologically proven in an occasion of a viral bout a few years earlier. Two days after the onset of facial palsy, elevated levels of specific anti-VZV IgG and IgM were observed by ELISA assay and complement fixation test. Small vesicles with typical VZV morphology appeared two days after the onset of the palsy at the triangular fossa and on the medial surface of the tragus, extending to the conchal area and the external meatus during the next 48 hours (Figure 1).

Pharmacological treatment with acyclovir (800 mg oral administration, five times per day) was started on postoperative day 15 (at the onset of the paresis) and continued for 10 days; eardrops had already been discontinued one week earlier and were not reintroduced. The eardrum looked normalized at micro-otoscopy, except for residual mild hyperemia around the malleus handle and the pars flaccida; pure tone audiometry revealed significant improvement in the air-conducted hearing threshold with reduction of the ABG within 20 dB HL and no sensorineural deficits.

An electroneuronography (EnOG), obtained 6 days after the onset of the palsy, showed complete denervation and an 80% decrease in nerve conduction by nerve excitability test. An electromyographic (EMG) study of the facial musculature on the 24th day after the onset confirmed the absence of response to stimulation at the stylomas-
Table 1. Systematic review of literature

<table>
<thead>
<tr>
<th>Author</th>
<th>Journal</th>
<th># of cases</th>
<th>Incidence</th>
<th>Age</th>
<th>Side</th>
<th>Onset time (days from surgery)</th>
<th>Type of surgery</th>
<th>Predisposing factors</th>
<th>Signs/symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Althaus SR et al.</td>
<td>Laryngoscope, 1973</td>
<td>5</td>
<td>0.22%</td>
<td>71, F</td>
<td>R</td>
<td>11</td>
<td>microdrill revision</td>
<td>CT sectioned</td>
<td>severe pain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(5/2307)</td>
<td>30, M</td>
<td>L</td>
<td>9</td>
<td>microdrill</td>
<td>CT sectioned</td>
<td>Otis media</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69, F</td>
<td>R</td>
<td>8</td>
<td>stapedectomy + vein</td>
<td>CT sectioned</td>
<td>Pain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47, M</td>
<td>L</td>
<td>13</td>
<td></td>
<td>CT sectioned</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42, M</td>
<td>R</td>
<td>5</td>
<td></td>
<td>CT sectioned</td>
<td>no</td>
</tr>
<tr>
<td>Storss LA et al.</td>
<td>Laryngoscope, 1983</td>
<td>1</td>
<td>0.2%</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Zohar Y et al.</td>
<td>J Laryngol Otol, 1985</td>
<td>1</td>
<td>n.s.</td>
<td>32, F</td>
<td>R</td>
<td>8</td>
<td>microdrill + vein</td>
<td>CT sectioned / surgical stress</td>
<td>Numbness, normal otoscopy</td>
</tr>
<tr>
<td>Shea JJ</td>
<td>J Laryngol Otol, 1988</td>
<td>1</td>
<td>0.1%</td>
<td>n.s., F</td>
<td>n.s.</td>
<td>5.5</td>
<td>microdrill + vein</td>
<td>IMMUNE RESPONSE IN THE FACIAL NERVE</td>
<td>n.s.</td>
</tr>
<tr>
<td>Bonkowsky V et al.</td>
<td>Ann Otol Rhinol Laryngol 1998</td>
<td>2 (out of 7)</td>
<td>0.11%</td>
<td>49, F</td>
<td>n.s.</td>
<td>7</td>
<td>microdrill + vein</td>
<td>(5 were tympanoplasty)</td>
<td>no</td>
</tr>
<tr>
<td>Glasscock and Shambaugh</td>
<td>Surgery of the ear, Saunders eds. 1990</td>
<td>2</td>
<td>&lt;1%</td>
<td>n.s., F</td>
<td>n.s.</td>
<td>&lt;10</td>
<td>microdrill + vein</td>
<td>Edema of the Fallopian canal</td>
<td>n.s.</td>
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<tr>
<td>Smith MC et al.</td>
<td>J Laryngol Otol, 1990</td>
<td>6</td>
<td>0.5%</td>
<td>29, M</td>
<td>n.s.</td>
<td>10</td>
<td>microdrill + vein</td>
<td>no</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40, M</td>
<td>n.s.</td>
<td>4</td>
<td>microdrill revision</td>
<td>no</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>44, M</td>
<td>n.s.</td>
<td>7</td>
<td>Exposed facial nerve</td>
<td>no</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>51, M</td>
<td>n.s.</td>
<td>4</td>
<td>no</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50, M</td>
<td>n.s.</td>
<td>6</td>
<td>no</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22, F</td>
<td>n.s.</td>
<td>4</td>
<td>no</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Wiet RJ</td>
<td>Otolaryngol Clin North America, 1993</td>
<td>1</td>
<td>n.s.</td>
<td>n.s., F</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ng and Maceri</td>
<td>American Journal of Otology, 1999</td>
<td>2</td>
<td>0.5%</td>
<td>54, F</td>
<td>R</td>
<td>5</td>
<td>KTP laser</td>
<td>no</td>
<td>n.s.</td>
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<tr>
<td>Shea JJ &amp; Ge X</td>
<td>Otol Neurotol, 2001</td>
<td>11</td>
<td>0.51%</td>
<td>mean age 8L, 53 (31–71, 3 R (5F, 6M)</td>
<td>7</td>
<td>3 microdrill, 8 Argon laser (6/11 were revisions)</td>
<td>5 with dehiscent facial canal, 2 CT sectioned / stretched o manipulated, 1 granulomatous reaction to gelfoam, 1 fever blister, 1 sinusitis</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Marioni G. et al.</td>
<td>ORL J Otorhinolaryngol Relat Spec, 2002</td>
<td>1</td>
<td>n.s.</td>
<td>59, M</td>
<td>R</td>
<td>7</td>
<td>microdrill</td>
<td>no</td>
<td>n.s.</td>
</tr>
<tr>
<td>Salvini F. et al.</td>
<td>Am J Otolaryngol, 2004</td>
<td>7</td>
<td>1%</td>
<td>61, F</td>
<td>R</td>
<td>10</td>
<td></td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Cohen M et al.</td>
<td>Otol Neurotol, 2010</td>
<td>2</td>
<td>0.4%</td>
<td>n.s., F</td>
<td>n.s.</td>
<td>12</td>
<td>microdrill</td>
<td>no</td>
<td>n.s.</td>
</tr>
<tr>
<td>Révész P et al.</td>
<td>Case Reports in Medicine, 2014</td>
<td>2</td>
<td>1.3%</td>
<td>52, F</td>
<td>R</td>
<td>8</td>
<td>KTP laser</td>
<td>no</td>
<td>Paraguesia and pain, slight pain</td>
</tr>
</tbody>
</table>

*3 days before DFP onset; n.s. = not specified; n.p. = not performed
A surgical re-exploration of the middle ear was not deemed useful nor was the decompression of the Fallopian canal up to its labyrinthine segment through a combined approach, as proposed in the past by some authors [26].

Eight weeks later, the facial palsy gradually started to improve, and at six months, it had returned to H-B grade III. At that time, a control EMG showed a significant increase of active motor units of the VII nerve. The facial weakness further improved in the following 8

### Author Positive history (recent labial HSV /previous VZV) HSV-HZV serology H-B grade at onset Therapy Recovery time (days) Final H-B grade

<table>
<thead>
<tr>
<th>Author</th>
<th>Positive history (recent labial HSV /previous VZV)</th>
<th>HSV-HZV serology</th>
<th>H-B grade at onset</th>
<th>Therapy</th>
<th>Recovery time (days)</th>
<th>Final H-B grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n.s.</td>
<td>Bacterial</td>
<td>Vasodilators, antibiotics</td>
<td>28</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>Complete</td>
<td>Nicotinic acid</td>
<td>120</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>Incomplete</td>
<td>-</td>
<td>22</td>
<td>incomplete</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>Complete</td>
<td>Steroids, vasodilators</td>
<td>56</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Storss LA et al. [29]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Zohar Y et al. [27]</td>
<td>n.s.</td>
<td>negative</td>
<td>Complete</td>
<td>Steroids (prednisolone)</td>
<td>30</td>
<td>I</td>
</tr>
<tr>
<td>Shea JJ [21]</td>
<td>n.s.</td>
<td>complete</td>
<td>Steroids</td>
<td>quick</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Bonkowsky V et al. [8]</td>
<td>n.s.</td>
<td>5 HSV1 (IgM in 1) - 2 n.p.</td>
<td>Steroid + acyclovir</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Glasscock and Shambaugh [11]</td>
<td>n.s.</td>
<td>Steroids</td>
<td>1 to several weeks</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith MC et al. [22]</td>
<td>n.s.</td>
<td>Incomplete</td>
<td>Steroids</td>
<td>11</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Wiet RJ [23]</td>
<td>n.s.</td>
<td>Incomplete</td>
<td>Steroids</td>
<td>11</td>
<td>I</td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>HSV, VZV</td>
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<td>Mills et al. [20]</td>
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<td>n.p.</td>
<td>Steroids (prednisone)</td>
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<td>II</td>
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<td>n.p.</td>
<td>and antiviral</td>
<td>&gt;10</td>
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Eight weeks later, the facial palsy gradually started to improve, and at six months, it had returned to H-B grade III. At that time, a control EMG showed a significant increase of active motor units of the VII nerve. The facial weakness further improved in the following 8
months, when it reached grade II, then stabilized with minimal resid-
ual dynamic dysfunction and slight oculo-oral synkinesis.

DISCUSSION
Typical RH was first described by James Ramsay Hunt in 1907; the
viruses can be reactivated during periods of generic temporary de-
pression of the immune system induced by physical or emotional
stress, concurrent bacterial or viral infections, neoplasms, mechanical
or surgical trauma, including local surgical stress. In 1972, Steffen
and Shelby firstly described an “Atypical RH Syndrome,” starting
with vague symptoms and without the typical vesicular eruption in
the concha (“Zoster sine herpete”). This clinical pattern is generally
extremely rare but it might proceed with the involvement of other
dermatomes or districts of the neck and face. To our knowledge,
this is the first case of DFP with typical RH syndrome after stapedecto-
my reported in the literature; the appearance of the pathognomonic
herpetic vesicles within few days after the onset of the palsy allowed
us to confirm unequivocally the pathogenetic role of VZV reactiva-
tion. In literature, RH with DFP has been reported only in two cases,
both after acoustic neuroma surgery. In particular, Gianoli et al.
described a case of DFP with typical RH after trans-labyrinthine re-
section of an acoustic neuroma: the herpetiform lesions appeared on
the ipsilateral ear canal and extended to the ipsilateral buccal muco-
sa; anti-VZV antibodies were elevated.

In DFP, an isolated increase in serum anti-VZV IgG antibodies has been
reported in literature confirming the suspect a viral reactivation. However, because most patients with breakthrough VZV will have pre-
existing elevated IgG antibody titers to VZV, conventional IgG determin-
ations are of limited value unless measurements are performed both
during the acute and convalescent phase. Evidence of VZV-specific
IgM in serum indicates recent exposure to VZV but does not discrimi-
nate between primary infection, reinfection, and reactivation. Moreover, the absence of IgM antibody does not exclude a recent VZV expos-
ure because IgM antibodies are inconsistently observed even among
cases with PCR-confirmed infection by VZV and the most reliable and
sensitive laboratory method for confirming varicella is the detection
of VZV DNA in samples (vesicular swabs, scabs, and saliva) obtained
from skin lesions using PCR. Unfortunately, this examination is not possible in DFP cases because typical RH is absent and none has been
reported data from saliva in the literature.

Furthermore, although the etiopathogenetic role of herpes virus re-
activation is controversial, serologic tests were obtained only in less
than half of the cases (21/48) of DFP reported in the literature. Even
positive history of recurrent labial HSV lesions or previous VZV infec-
tion, which is considered valuable anamnestic indicator, were often
underestimated or not even investigated.

Alternatively, the incidence of DFP after stapedectomy ranges be-
tween 0.07% and 1.4% in the literature, corresponding to 1/450 to
1/1000 of operated ears. A higher incidence has been reported in more limited series, (1.4%–37.5%), particularly those employing
lasers. Considering the possible reduction of natural immunity following
the mass VZV vaccination campaign, one might expect a recrudes-
cence of VZV after stapedectomy; instead, a relevant increase in the
incidence of DFP has not yet been observed.

When evident, typical conchal blebs of RH syndrome appear later
than the facial paresis; electrophysiological tests do not differenti-
ate post-surgical DFP from other types of facial paresis; and imag-
ing after uncomplicated stapedectomies with DFP is not conclusive,
although useful for possible medicolegal purposes to exclude iatro-
genic lesions. Thus, presence of specific anti-VZV IgG and IgM should
be checked for confirmation of etiology; special attention must be
paid to prodromal symptoms, such as unexplained late pain (acute
neuropathic otalgia).

In conclusion, “Zoster sine herpete” is the most frequent pattern re-
ported in literature and reactivation of VZV might be underestimated
in clinical practice because of the lack of typical RH in DFP after sta-
pedectomy.

Given the paucity of DFP, the universal preventive use of antivirals
in stapes surgery is not warranted, except for selected cases. For this
reason, the initial intense pain might indicate those cases who would
benefit from immediate administration of antivirals.
Ethics Committee Approval: Ethics committee approval was received for this study from the internal ethical committee.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES

Electrophysiological and Histopathological Evaluation of Effects of Sodium-2 Mercaptoethanesulfonate Used for Middle Ear Surgery on Facial Nerve Functions

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OBJECTIVE: Sodium-2-mercaptoethanesulfonate (MESNA) is widely used in medicine because of its antioxidant and mucolytic effects. In recent years, it has been used in otologic surgery. Because it cleaves disulfide bonds, it is used to easily dissect the epithelial matrix in cholesteatoma and atelectasis. In this study, we hypothesized that MESNA does not have any toxic effect on the facial nerve, and the effects of MESNA on the facial nerve were examined histologically and electrophysiologically.

MATERIALS and METHODS: Twenty Wistar albino rats were used. Groups A and B were designated as the control and sham groups, respectively. The animals in groups C and D were administered 20% and 50% of MESNA solution, respectively, after the facial nerve was exposed in the parotid region. Electromyography (EMG) measurements were performed preoperatively and postoperatively at 4 weeks. The animals were subsequently euthanized; facial nerve samples were taken for histopathological examination.

RESULTS: When EMG parameters were compared within and between each group, preoperative and postoperative results were not statistically significantly different. Histopathological examination showed that MESNA did not cause any inflammation, granulation tissue, or foreign body reaction.

CONCLUSION: To the best of our knowledge, the effects of MESNA on facial nerve functions have not been investigated. In this study, the effects of MESNA after direct application to the facial nerve were examined electrophysiologically and histologically, and it was determined that MESNA did not cause any toxic effects. It was concluded that MESNA can, therefore, be safely used during middle ear surgery.

KEYWORDS: MESNA, rat, electromyography, histopathology

INTRODUCTION
Cholesteatoma is a common pathology in chronic otitis media (COM). Residual cholesteatoma and late-term disease recurrence are major problems related to COM surgery [1-3]. Different applications may be used to reduce the incidence of residual cholesteatoma. Oto-endoscopy systems have been introduced for providing better visibility and eradicating “blind spots” [4]. The use of a potassium titanyl phosphate laser provides more effective matrix dissection [5]. In addition, the application of galectin-7 using an immunofluorescence technique enabled visualization of the microscopic epidermal remains [6].

Although new techniques and surgical procedures have been introduced, the incidence of residual cholesteatoma was reported to vary from 5% to 50% [7]. The cholesteatoma matrix is mainly covered with keratin, and it contains numerous disulfide bonds. Chemical separation of these bonds allows dissection to be performed more easily and effectively during surgery [8]. Sodium-2-mercac-
toethanesulphonate (MESNA), a sodium salt of 2-thiosulphonate an-
ion, is now used for this purpose during otologic surgery; it provides
more effective dissection because it cleaves the disulfide bonds [9]. In
addition, because MESNA is a synthetic sulfur compound, it enables
separation of mucus polypeptide chains by mucolysis of the disulfide
bonds [9]. Moreover, owing to its antioxidative, protective, and mucol-
ytic features, it is commonly used in medicine [10]. As a thiol-based
cytotrophic agent, it is used for prophylactic treatment in cyclo-
phosphamide-induced hemorrhagic cystitis. Because of its mucolic-
ytic effects, it is also used in respiratory medicine (in the treatment
of asthma, chronic bronchitis, pharyngolaryngitis, etc.) as well as in
some surgical treatments, for example, ear, nose, and throat, ortho-
pedic, and gynecological fields [11].

Previous studies have reported that the use of MESNA in atelectatic
and adhesive middle ear and cholesteatoma surgery is not associat-
ed with ototoxic effects and had neuroprotective effects; however,
to the best of our knowledge, its direct effect on the facial nerve has
not been demonstrated [9-12]. The possibility of dehiscence develop-
ment in the fallopian canal of the facial nerve is very high in the
presence of pathologies progressing with dissolving bone structures,
such as cholesteatoma; the use of MESNA for separating the choles-
etoma matrix in such surgeries inevitably results in contact with
the facial nerve, directly or by passing through bony dehiscence. In
this study, we investigated the effects of MESNA, which is commonly
used during middle ear surgery, on the facial nerve using electro-
physiological and histopathological methods.

MATERIALS and METHODS

This study was approved by the local ethics committee on animal ex-
periments (No.01.11.2016-504). Twenty male Wistar rats raised in the
animal experiments laboratory, aged 12-14 weeks and weighing 250-
300 g were used in this study. Animals were placed in cages with free
access to food and water. The cages had 50±10% relative humidity
at 23°C±3°C and artificial lighting was made available from 8:00 a.m.
to 8:00 p.m. The rats were equally divided into four groups. Group A
was the control group and group B constituted the sham group. After
identifying the facial nerve, the animals in group C were given 20%
MESNA solution and those in group D were given 50% MESNA solu-
tion. In this study, MESNA (Ureomitexan, MESNA, Eczacıbaşı-Baxter,
Turkey) was diluted with physiological saline to obtain 20% and 50%
solutions; a single dose was administered to the animals.

The experiments were performed only on the left facial nerve of the
animals; electromyography (EMG) recordings were obtained. The rats
were given general anesthesia preoperatively and postoperatively at
4 weeks using intramuscular ketamine hydrochloride (50 mg/kg) and
xylazine hydrochloride (5 mg/kg), and EMG was recorded to obtain
combined muscle action potentials from the mandibular branch of
the facial nerve using PowerLab 26T multiple recording devices and
LabTutor (ADInstruments Pty Ltd.). Latencies, amplitude, wave dura-
tion, and supramaximal alert thresholds values were recorded from
the test results and compared between the study and control groups.

Electromyography recordings were obtained according to the model
described by Salomone et al. [13]. Specially designed bipolar subdermal
needle electrodes were used for stimulation and records during EMG.
These monopolar electrodes had a thickness of 0.35 mm and length of
12 mm, and they were placed 5 mm apart from each other with a 3-mm
gap at the tip for subcutaneous entry and the remaining 9 mm was
taped for insulation. Similarly, a monopolar needle electrode wrapped
with 9-mm tape was used for grounding. The recording electrode was
placed subdermally, 3 mm away from the corner of the mouth, parallel
to the lower lip. The stimulation electrode was placed subdermally, 25
mm away from the corners of the mouth, with the anode (+) located
proximally and the cathode (-) located distally to stimulate the main
body of the facial nerve. The ground electrode was placed subdermally
midway between these two electrodes. For electrical stimulation, the
stimuli given started at 0.5 mA and were increased to 5 mA in 0.5-mA
increments. The duration of each stimulus was 50 ms. After the latency
stimulus was given, time until the first wave was observed over the
isoelectric line (in ms), distance between the positive and negative
wave amplitude peaks (in millivolts), and time between the start and
end of the wave–wave duration (in ms) were calculated. Supramaximal
stimulation thresholds were determined to be the threshold required
for stimulation of all fibers. For comparison at a constant value, the re-
cords obtained by giving 2.5 mA which was the appropriate stimulus
based on average supramaximal stimulus threshold, were evaluated.

Following preoperative EMG measurements, five rats were separated
as the control group without any intervention (group A). In all other
animals, the left parotid region was entered subcutaneously using
an approximately 2-cm skin incision, and the facial nerve trunk was
located on the parotid gland with blunt dissection (Figure 1). Group B
was designated as the sham group, and the operation was termi-
nated in five animals, closing the wound with 4/0 Vicryl without any
additional intervention. Animals in group C were administered 20%
MESNA solution after the facial nerve was located, and the wound
was closed after waiting for approximately 5 min. Five animals in
group D were administered 50% MESNA solution onto the facial
nerve for 5 min, and the operation was subsequently terminated,
closing the wound.

After EMG measurements were performed under anesthesia postop-
erratively at 4 weeks, all rats included in the study were euthanized
by guillotine decapitation, and sufficient amounts of facial nerve and
surrounding tissue samples were obtained from the region of inter-
vention. Each tissue sample was fixed in formaldehyde solution for
24 h (10%) and embedded in paraffin blocks for histopathological ex-
amination. Sections of 4-6 micron thickness were obtained in pathol-
ogy laboratories using a microtome. The sections were stained using
hematoxylin and eosin and S-100 immunohistochemistry stains (S-
100, Biocare Medical, USA) and evaluated by a single pathologist un-
der a light microscope (Olympus BX53) to be photographed at 40x,
100x, and 200x magnification fields. All histopathological micromea-
surements were quantitatively made using an Olympus DP73 cam-
era and digital microscopy software; each parameter was individually
classified as follows:

- Foreign body reaction (Yes/No)
- Inflammation (None: 0, Mild: +, Moderate: ++, Extensive: ++++, and
  Widespread inflammation and necrosis: +++++)
- Granulation tissue (Yes/No)
- Status of the perineural sheath and myelin structure (myelin struc-
ture preserved/irregular myelin structure)
- Axonal degeneration (Yes/No)
All samples were assessed by a single pathologist using a protocol blinded to the type of treatment provided to each group.

**Statistical Analysis**

Statistical analysis was performed using the Statistical Package for Social Sciences version 22 software (IBM Corp.; Armonk, NY, USA). For the evaluation of test results, preoperative and postoperative mean and standard deviations were calculated for latencies, amplitude, wave duration, and supramaximal stimulus thresholds in the study and control groups. Histopathological data were evaluated together with the statistical results. For comparison of quantitative data between the groups, the Kruskal-Wallis test was used for data not normally distributed, and the Wilcoxon sign test was used for in-group comparisons. A p value of <0.05 was considered to be statistically significant.

**RESULTS**

One animal in group C (MESNA 20%) was excluded from the study because it died because of anesthesia provided before preoperative EMG measurements.

When the wave amplitude, as measured by EMG, was evaluated between the groups, there was no statistically significant difference between preoperative and postoperative amplitude levels of any of the groups (p>0.05). Compared with the preoperative amplitude level, there was no statistically significant change in the postoperative amplitude level of groups B, C, or D (p=0.893, p=0.068, or p=0.500, respectively, Table 1).

In terms of wave latency levels, as measured by EMG, no statistically significant difference was noted in preoperative and postoperative EMG measurements (Table 2). The wave duration levels were also similar between the groups (Table 3). The supramaximal stimulus thresholds did not show any statistically significant differences between the groups (Table 4).
tive measurements between the groups (p>0.05). Compared with the preoperative latency level, there was no statistically significant change in the postoperative latency level of groups B, C, or D (p=0.068, p=0.713, or p=0.496, respectively, Table 2).

Table 3 denotes the evaluation of wavelength within each group and between all groups. Accordingly, there was no statistically significant difference in the preoperative and postoperative wavelengths between the groups (p>0.05). The increase noted in the wavelength in the postoperative period compared with that in the preoperative period did not show statistically significant difference in groups B, C, or D (p=0.051, p=0.068, or p=0.345, respectively).

When the preoperative and postoperative supramaximal stimulus threshold levels, as measured by EMG, were evaluated between study groups, no statistically significant difference was detected (p>0.05). Compared with the preoperative stimulus threshold level, there was no statistically significant change in the postoperative level in groups B, C, or D (p=0.068, p=0.157, p=1.000, respectively, Table 4).

Table 5 shows the results of histopathological examination. The degree of inflammation in all groups was 0. Foreign body reaction and granulation tissue were not detected in any of the groups. Perineural sheath and myelin structure were normal in all groups, and axonal degeneration was not observed (Figure 2-4).

**DISCUSSION**

MESNA is the sodium salt of 2-thiosulphonate anion. It is widely used in medicine because of its preservative, antioxidant, and mucolytic properties [12]. It is used as a cytoprotective agent to prevent hemorrhagic cystitis and as a mucolytic agent to improve pulmonary functions. In addition, the sulfhydryl group has the potential to clean the reactive oxygen metabolites and is used as a systemic protective agent against chemotherapy toxicity [9, 11].

In the field of otology, MESNA is used to easily dissect the tissue layers during ear surgeries, such as atelectatic ear and cholesteatoma, and its use has increased in recent years [7]. Some animal studies have shown that MESNA has no harmful effects on the cochlea and does not cause ototoxicity [8, 12]. However, the possibility of dehiscence development...
not indicate any difference in terms of inflammation, foreign body reaction, or granulation tissue formation between the groups, and it was observed that the perineural sheath and myelin structure were preserved in all nerves without any axonal degeneration.

Our study has some limitations. The extratemporal portion of rats’ facial nerves was evaluated in this study. MESNA was applied subcutaneously by dropping onto the facial nerve, and after waiting 5 min, the skin and subcutaneous tissue were closed. Through the middle ear, the facial nerve was located in a bone canal. During middle ear surgeries, leakage of MESNA into the dehiscent facial canal can increase the pressure on the nerve, and facial paralysis may occur following edema and inflammation. Because this study was unable to evaluate the part of the facial nerve positioned in the middle ear bone canal and because there was lack of knowledge regarding the potential for functional problems after prolonged exposure, studies are needed to investigate the neurotoxic effect of MESNA during middle ear surgery. To the best of our knowledge, this is the first study to investigate the effects of MESNA during otologic surgery, which enables cleavage of disulfate bonds and therefore, allows easy and safe dissection, particularly in cholesteatoma and adhesive otitis media, on the facial nerve in the parotid region by physiological and histopathological methods. The findings suggest that a single application of MESNA does not cause any histopathological and electrophysiological changes that indicate toxicity on the facial nerve.

CONCLUSION
To the best of our knowledge, this is the first study to investigate the effects of MESNA during otologic surgery, which enables cleavage of disulfate bonds and therefore, allows easy and safe dissection, particularly in cholesteatoma and adhesive otitis media, on the facial nerve in the parotid region by physiological and histopathological methods. The findings suggest that a single application of MESNA does not cause any histopathological and electrophysiological changes that indicate toxicity on the facial nerve.

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Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

In this study, the effects of MESNA on facial nerve functions were evaluated using an animal model and based on electrophysiological and histopathological findings. The facial nerve in the parotid region of the rats was preferred because it is easy to reach and has the same characteristics as the facial nerve in the middle ear. As observed in previous studies on MESNA, a single dose of solutions diluted using physiological saline at concentrations of 20% and 50% was tested on separate groups. The wavelength, latency, amplitude, and supramaximal stimuli threshold levels, as measured by EMG, were recorded and statistically evaluated within each group as well as between all groups. When the preoperative and postoperative wavelengths, latencies, amplitudes, and supramaximal stimulus threshold levels were evaluated between study groups, no statistically significant difference was detected (p>0.05). Compared with all preoperative findings, there was no statistically significant change in the postoperative findings of groups B, C, or D. Histopathological examination did by means of mesna in cholesteatoma surgery.
A Clinical Trial of Proton Pump Inhibitors to Treat Children with Chronic Otitis Media with Effusion

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OBJECTIVE: Gastroesophageal reflux (GER) is considered a cause of otitis media with effusion (OME). This study aimed to investigate whether OME can be effectively treated with a proton pump inhibitor (PPI), therefore implicating GER as a causative factor of OME.

MATERIALS and METHODS: A PPI or placebo was randomly administered to enrolled subjects for 4–8 weeks. To monitor effusion status, subjects underwent monthly pneumatic otoscopy and acoustic reflectometry. At enrollment and at completion of treatment, subjects underwent an audiogram and tympanogram for assessing changes in hearing due to altered fluid levels in the middle ear. After the treatment period, tympanostomy tube placement was recommended for subjects with unresolved effusion.

RESULTS: This study enrolled 16 patients with an average age of 5.17 years. Between the treatment and placebo groups, there was no significant difference in the need for tympanostomy tubes. At completion of this study, patients receiving Lansoprazole demonstrated a significant improvement in pure tone average (p<0.01) and speech recognition thresholds (p=0.04). Four patients (25%) from the cohort dropped out of the study. Eight patients (50%) from the cohort required tympanostomy tube placement.

CONCLUSION: Owing to difficulties with recruitment and small sample size, this study was unable to demonstrate the use of PPI in treating OME. A larger study is needed for further evaluation of this process.

KEYWORDS: Otitis media, effusion, proton pump inhibitor

INTRODUCTION

Otitis media with effusion (OME) is a common childhood disorder, and its true incidence is difficult to ascertain because it is asymptomatic by definition. It is estimated that up to 25% of school-aged children have an effusion at some point [1]. Due to the frequency of episodes of acute otitis media during the early years of life, a young child may spend a significant portion of those years with effusion and associated hearing loss. OME is particularly important because hearing impairment associated with middle ear effusion over prolonged periods of time during the first few years of life may affect the development of speech and language. Children may receive decreased, distorted, or inconsistent auditory signals due to OME, which adversely affects the development of speech. In addition, impaired hearing can lead to the “tuning out” of sounds, later interpreted as inattentiveness and distractibility in the classroom [2]. In children, persistent middle ear effusion (>3 months) is the most common cause of hearing loss, and it is commonly treated with tympanostomy tube placement [3].

The three primary factors associated with the development of OME are upper respiratory tract infections, insufficient ciliary clearance, and poor drainage of the Eustachian tube [4]. The reflux of gastric contents, i.e., gastroesophageal reflux (GER), may contribute to the inflammation that is central to both the development and persistence of OME. GER, like OME, is common in children. GER has been shown to play a causative role in multiple upper respiratory tract complaints, most commonly intermittent vomiting and regurgitation in children. The most common extra-digestive symptoms are related to the respiratory tract, and they include bronchial hyperactivity, chronic cough, laryngitis, hoarseness, recurring pneumonia, otitis, and sinusitis [5]. In 2002, Tasker et al. [6] reported the presence of pepsin/pepsinogen in 90.8% of 65 middle ear effusion (MEE) samples obtained at the time of myringotomy in children. The pepsin–pepsinogen levels in these samples were 1000 times higher than those in the serum. Pepsin was noted to be active in 14.4% of 152 subjects who underwent tympanostomy tube placement [8]. Crapko et al. [7] identified pepsin activity in the mid-
dle ear fluid of 60% of 20 subjects undergoing tympanostomy tube placement. These two studies suggest that GER plays a role in the development of OME. Esophageal pH monitoring with double probe confirmed that acid may pass the anatomical barrier of the upper esophageal sphincter and come into contact with the laryngeal and hypopharyngeal mucosa [8]. Contencin and Narcy have postulated that reflux material could also reach the nasopharynx. The angle of the pediatric Eustachian tube may allow the reflux of gastric contents into the nasopharynx to enter the middle ear. The putative mechanism of the development of OME in the setting of GER is through contact of the nasopharynx with reflux material [8]. Repeated exposure of the ciliated respiratory epithelium to pH 4 or less prevents ciliary movement and clearance. Hydrochloric acid and pepsin cause local inflammation, edema, and ulceration of the respiratory mucosa, leading to the loss of Eustachian tube ventilatory function.

Current standards of treatment for GER include the use of a proton pump inhibitor (PPI) such as lansoprazole or omeprazole [10]. These medications have been shown to be safe for use in children as young as 2 years of age. Reflux reduction or the use of PPIs may have a role in the treatment of OME [11]. McCoul et al. [12] demonstrated in their 2011 before-and-after intervention study that following treatment with antireflux therapy, children with OME and GERD demonstrated an improved quality of life. Their study employed validated measures of disease burden of OME and GERD and demonstrated improvement with antireflux therapy. They concluded that a reduction in GER may play a role in the prevention of otitis media [12]. Thus, so far, the association between GER and OME has been difficult to establish because most children with GER or OME are asymptomatic. The purpose of the present investigation is to discern whether OME can be effectively treated with PPI, thus implicating the role of GER in the development and persistence of OME.

MATERIALS and METHODS
This randomized double-blind placebo-controlled study was conducted at a tertiary-care pediatric otolaryngology practice. Institutional review board approval was provided by the Institutional Human Research Protection Office. After parental informed consent was obtained, children aged 2–12 years with a history of chronic OME who had met the indications for tympanostomy tube placement were recruited in the study. OME is defined as the presence of middle ear fluid on physical examination for at least 3 months, in at least one ear, and audiogram or hearing screening suggesting conductive hearing loss. Subjects were recruited from the clinical practice of a tertiary pediatric otolaryngology care center. Patients with a medical history or concurrent conditions known to increase the incidence of otitis media, OME, or GER, including cleft palate, neurologic delay, or Down syndrome, were excluded. In addition, patients with structural abnormalities of the tympanic membrane, including atelectasis, cholesteatoma, or deep retraction pockets, were also excluded. Randomization, which was performed by the research pharmacist who dispensed the study medications, was performed in blocks of six using a coin-flip to ensure equal numbers of patients in each group.

The treatment arm was given the PPI lansoprazole for 4–8 weeks, whereas the placebo arm was given placebo (sugar pill). Two dosages of lansoprazole were administered based on the weight of the patient. Patients weighing less than 30 kg were given 15 mg lansoprazole per day, whereas patients weighing more than 30 kg were given 30 mg per day. An investigational drug application was filed with the FDA, and exemption was subsequently granted. Each month, the patients returned to the clinic for an otologic examination to monitor the presence of fluid in the middle ear. The medication was administered for a minimum of 4 weeks, barring any change to the tympanic membrane necessitating urgent tympanostomy tube placement. The study included three clinic visits: an enrollment visit, followed by a visit after 4 weeks of treatment, and a third visit after 8 weeks of treatment. At any time along the experimental timeline, parents had the option of stopping drug treatment for tympanostomy tube placement. In addition, after 8 weeks of treatment with medication (either placebo or PPI), the parents were given the option to continue medication use for 4 more weeks, with one additional follow-up visit at 12 weeks. At the time of enrollment in the study, the patients underwent audiogram with tympanometry. These were repeated at the conclusion of the 3-month study period, if the effusions remained, to monitor changes in hearing due to altered fluid levels in the middle ear. At each clinic visit, the parents of the patient completed a validated questionnaire regarding the presence of symptoms associated with GER, the Gastroesophageal Reflux Questionnaire (I GERQ-9) [13]. At the conclusion of the treatment period, tympanostomy tube placement was recommended for patients with unresolved effusion.

The primary outcome of this study was to double the resolution rate of 20% in 3 months without intervention to 40% in 3 months with the use of PPI. Using a one-tailed alpha level of 0.05 to determine statistical significance, the minimum number of subjects that we had initially aimed to recruit was 64; in this calculation, a 10% drop-out rate was assumed.

Symptoms of GERD were monitored using the I GERQ-9. The I GERQ-9 is a validated questionnaire developed to improve history taking of infants and toddlers with suspected GER. The questions cover demographics, symptoms, and possible causes that are answered by the caregiver. The GER3-9P is used for older children. It is also a validated questionnaire for children aged 3–7 years that asks caregivers about current symptoms suggestive of GER.

Before the start of the treatment as well as at the end of the 2-month treatment period, tympanograms were performed for each patient. Tympanogram results from enrollment and study completion were compared and analyzed using a t-test. Audiograms were performed only for those patients for whom it was deemed clinically necessary.

Statistical Analysis
Statistical analysis was performed using Statistical Packages for the Social Sciences for Windows, version 21 (IBM Corp.; Armonk, NY, USA) and Microsoft Excel (Redmond, WA, USA). A two-sided binomial test was used to compare the presence and absence of effusions and hearing loss. Hearing loss was defined as a pure tone average (PTA) >25 dB. Fisher’s exact test or chi-square test was used to examine variables with discrete values associated with the outcome of interest, including demographic, family history, and comorbidity variables. Student’s t-test was used to examine the association of continuous variables with the outcome.
RESULTS
A total of 16 patients (10 males, 62.5%) met the inclusion criteria. The study aimed to recruit a minimum of 64 patients to establish significance allowing for a 10% drop-out rate. This unfortunately proved to be very difficult because this study was performed at a tertiary-care center and a majority of the patients encountered had other health problems leading to their exclusion. The study was closed after 2 years due to the inability to recruit subjects.

The mean (SD) age of the patients was 5.17 (1.72) years (Table 1). Fourteen children (88% of the cohort) had middle ear effusion at the time of enrollment. Similarly, 14 patients had at least one type B tympanogram at the time of enrollment. The most common GERD symptoms were halitosis, headache, coughing while lying down, difficulty breathing while sleeping, and itching of the ear (6 (37.5%)) (Table 2). The study participants belonged to households where the primary caregiver had an average educational level of an associate's degree and an average household income of $70,000–$79,999 per year. Additional characteristics are presented in Table 1.

Patients were randomly assigned to receive either Lansoprazole Solutabs or placebo. At the time of enrollment, the placebo group had significantly more exposure to second-hand smoke (p=0.02) and had significantly more pets at home (p=0.04) (Table 1). Of those who completed the study, the placebo group had a significantly greater history of streptococcal infection (p=0.03) and continued to have a significantly greater exposure to second-hand smoke (p=0.01). Those in the placebo group were significantly more likely to have at least one otalgia per week (p=0.01). There was no significant difference in the need for tympanostomy tubes between the treatment group and the placebo group. At completion of the study, the patients receiving Lansoprazole demonstrated a significant reduction in diarrhea (p=0.03) and halitosis (p=0.03) and an improvement in hearing.

Table 1. Baseline demographic characteristics of 16 study patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cohort n=16 (%)</th>
<th>Placebo n=7 (%)</th>
<th>Lansoprazole n=9 (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (sd)</td>
<td>5.17 (1.7)</td>
<td>5.53 (1.2)</td>
<td>4.89 (2.0)</td>
<td>0.49</td>
</tr>
<tr>
<td>Male</td>
<td>10 (63)</td>
<td>6 (85.6)</td>
<td>4 (44.4)</td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>2 (13)</td>
<td>0</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Breast-fed</td>
<td>11 (69)</td>
<td>4 (57.1)</td>
<td>7 (77.8)</td>
<td></td>
</tr>
<tr>
<td>Needed PETs prior to the study</td>
<td>11 (69)</td>
<td>5 (71)</td>
<td>6 (67)</td>
<td></td>
</tr>
<tr>
<td>Chronic medical problem</td>
<td>3 (19)</td>
<td>1 (14.3)</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Allergies</td>
<td>2 (13)</td>
<td>1 (14)</td>
<td>1 (14)</td>
<td></td>
</tr>
<tr>
<td>History of tonsillectomy and adenoidectomy</td>
<td>2 (13)</td>
<td>1 (14.3)</td>
<td>1 (11.1)</td>
<td></td>
</tr>
<tr>
<td>History of otitis media in the lifetime, years, mean (sd)</td>
<td>1.81 (0.75)</td>
<td>2.1 (0.69)</td>
<td>1.56 (0.73)</td>
<td></td>
</tr>
<tr>
<td>Attends daycare</td>
<td>9 (56)</td>
<td>5 (71.4)</td>
<td>4 (44.4)</td>
<td></td>
</tr>
<tr>
<td>Attends school</td>
<td>11 (69)</td>
<td>4 (57.1)</td>
<td>7 (77.8)</td>
<td></td>
</tr>
<tr>
<td>Family history of otitis media</td>
<td>8 (50)</td>
<td>2 (28.6)</td>
<td>6 (66.7)</td>
<td></td>
</tr>
<tr>
<td>History of reflux</td>
<td>2 (13)</td>
<td>0</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Exposure to second-hand smoke</td>
<td>6 (38)</td>
<td>5 (71.4)</td>
<td>1 (11.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Has pets at home</td>
<td>12 (75)</td>
<td>7 (100)</td>
<td>5 (55.6)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 2. Physical examination and symptoms of study participants at the time of enrollment

<table>
<thead>
<tr>
<th>Physical Examination Finding/Symptom</th>
<th>Cohort n=16 (%)</th>
<th>Placebo n=7 (%)</th>
<th>Lansoprazole n=9 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart burn</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chest pain</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sour taste in mouth</td>
<td>2 (13)</td>
<td>0</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Coughing while supine</td>
<td>6 (38)</td>
<td>4 (57)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Increased salivation</td>
<td>3 (19)</td>
<td>1 (17)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Difficulty sleeping after eating</td>
<td>3 (19)</td>
<td>0</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Difficulty breathing while sleeping</td>
<td>6 (38)</td>
<td>2 (29)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Earache</td>
<td>8 (50)</td>
<td>5 (71)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Itching of the ear</td>
<td>6 (38)</td>
<td>3 (43)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Drainage from ears</td>
<td>2 (13)</td>
<td>1 (14)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Difficulty hearing</td>
<td>8 (50)</td>
<td>4 (57)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Ringing in the ears</td>
<td>5 (31)</td>
<td>4 (57)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Headache</td>
<td>6 (38)</td>
<td>4 (57)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4 (25)</td>
<td>0</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Bad breath</td>
<td>6 (38)</td>
<td>2 (29)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Syncopal episode</td>
<td>1 (6)</td>
<td>0</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Bloating</td>
<td>3 (19)</td>
<td>1 (14)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

PETs: Tympanostomy tubes

TM: tympanic membrane
Table 3. Audiometric characteristics of study participants at the time of enrollment and at completion of the study

<table>
<thead>
<tr>
<th></th>
<th>Placebo Enrollment dB HL</th>
<th>Placebo Completion dB HL</th>
<th>Lansoprazole Enrollment dB HL</th>
<th>Lansoprazole Completion dB HL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right pure tone average</td>
<td>26.2</td>
<td>30.8</td>
<td>26.4</td>
<td>36.7</td>
<td>0.00</td>
</tr>
<tr>
<td>Left pure tone average</td>
<td>23.1</td>
<td>12.5</td>
<td>26.7</td>
<td>8.3</td>
<td>0.00</td>
</tr>
<tr>
<td>Right speech recognition threshold</td>
<td>26.7</td>
<td>15</td>
<td>26.4</td>
<td>20</td>
<td>0.04</td>
</tr>
<tr>
<td>Left speech recognition threshold</td>
<td>21.7</td>
<td>20</td>
<td>27.9</td>
<td>7.5</td>
<td>0.04</td>
</tr>
</tbody>
</table>

as demonstrated by PTA (p=0.00) and speech recognition threshold (SRT) (p=0.04) (Table 3).

Eight (66%) of the twelve patients who completed the study required tympanostomy tubes. Those who required tubes were significantly more likely to have a family history of otitis media (p=0.01). The educational level of the primary caregiver was significantly lower for patients who required tympanostomy tube placement (p=0.03).

Four (25%) patients dropped out, failing to return for the second visit. All patients who had withdrawn had been assigned to the Lansoprazole group. They were significantly different from those who completed the study in two ways: those who had withdrawn were significantly less likely to have an abnormal position of the tympanic membrane (p=0.03) and were less likely to have tinnitus (p=0.01).

**DISCUSSION**

In developed nations, chronic OME is the most common cause of pediatric hearing loss [14]. Gastroesophageal reflux disease (GER) is considered a cause of OME. In pediatric sinusitis, improvement with empiric antireflux therapy has been demonstrated in up to 85% of children studied [16]. This has led to a decrease in the number of surgical procedures required for treating pediatric sinusitis [17]. We hypothesize that there is a similar pathogenesis relating OME with GER. However, current data measuring the concentration of pepsin and pepsinogen in middle ear fluid report conflicting results, and the association between GER and OME remains inconclusive. Recent literature indicates that pepsin present in middle ear effusions is almost certainly due to reflux of gastric contents and that there may in fact be a role of antireflux therapy for treating OME.

The present study intended to demonstrate whether OME can be effectively treated with PPIs, the current accepted treatment for GER, therefore implicating GER as a causative factor of OME. However, owing to difficulty with patient recruitment and small sample size, this could not be accomplished. Patients treated with PPI did show improvements in hearing over the 3-month period. However, there were no significant differences in the need for tympanostomy tubes at the end of the trial period. This is likely attributable to the small sample size. The number of subjects who completed the study was less than 20% of the initial intended number to establish significance. With greater enrollment, the administration of a PPI may lead to significantly improved hearing and reduction in the need for tympanostomy tubes. At the study conclusion, 66% of the participants required tympanostomy tubes. A total of 33% of our subjects achieved resolution of their effusion by the end of the 3-month study period. Rosenfeld et al. [18] reported a 28% spontaneous resolution rate of effusion at the end of 3 months (95% CI: 14%–41%), with respect to effusion of unknown duration, if left untreated. This suggests that the treatment of our participants, whether with placebo or Lansoprazole, made no significant difference in the course of disease as the resolution rate was nearly the same as that for untreated patients.

The small number of subjects may account for the statistically significant differences between the placebo arm and the treatment arm. The placebo group both at the time of enrollment and at the study conclusion was noted to have a significantly greater exposure to second-hand smoke in comparison to the treatment group. Paradise et al. noted in their 1997 study that exposure to second-hand smoke is a risk factor for the development of OME. This may have attributed to the persistence of the effusion beyond the 3-month treatment period of the present study. In addition, Paradise et al. [19] also cited a lower socioeconomic status as well as family history of OM as risk factors for the development of OME. In our study, patients with a family history of OME and those with a primary caregiver who had a lower educational level were significantly more likely to require tympanostomy tube placement at the study conclusion. This is in concordance with current literature on the topic.

As expected, the administration of PPIs led to reduction in gastrointestinal symptoms, including diarrhea and halitosis. Shashidhar et al. [20] demonstrated in their 2000 study that administration of PPIs significantly decreased the incidence of gastrointestinal symptoms, including vomiting, abdominal pain, diarrhea, anorexia, and halitosis. We would expect this result to reflect in our study cohort [20].

It was difficult not only to recruit patients who met the inclusion criteria but also to maintain study involvement. A total of 25% of recruited patients dropped out. All of them were in the placebo group. They were significantly less likely to have an abnormal position of the tympanic membrane as well as tinnitus at the time of enrollment. Although we cannot say definitively, this implies that patients who had withdrawn were less symptomatic in comparison to those who remained in the study. Those who had withdrawn possibly did not have
OME as severely as those who remained in the study. Undoubtedly, this would have an implication for study outcomes as the withdrawal of patients who were less severely affected would result in a higher than expected rate of tympanostomy tube placement at the study conclusion. If repeated, this study should be conducted in a community setting rather than a tertiary-care setting. It was very difficult to recruit patients without any other comorbidity from a tertiary-care setting.

Those who received PPI at the study conclusion had significantly improved PTA and SRT. Although our study was a small-scale study and could not prove significance in the rate of tympanostomy tube placement between the two groups, the improvement in audiological outcomes in those receiving PPIs implies that some reduction of effusion occurred. However, we cannot determine if this is the impact of the PPI or the natural healing process that occurs in OME resolution. This study should be repeated in a community-based otolaryngology practice with larger number of participants. In a large-scale study, effusions may be sufficiently reduced by the administration of PPIs to demonstrate a difference in tympanostomy tube requirement after 3 months.

Ethics Committee Approval: Ethics committee approval was received for this study from Washington University IRB was provided by the Institutional Human Research Protection Office in August 2007.

Informed Consent: Written informed consent was obtained from parents’ of the patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors have no financial disclosures to report.

REFERENCES

INTRODUCTION
The prevalence of tympanic membrane (TM) retraction in children has been reported to be 8%-10%, with most cases described as mild [1]. While some retractions are reflective of a transient negative pressure state in the middle ear, others become chronic. In pediatric patients, most retractions arise from the pars tensa, as opposed to the pars flaccida [2, 3]. When pars tensa retractions worsen, adherence to the medial tympanic wall and incus may occur. Once a retraction establishes contact with the ossicular chain, concern arises for the development of permanent hearing loss. However, little is known about the incidence of permanent hearing loss in such retraction cases. Similarly, the risk of cholesteatoma formation from a pars tensa retraction remains to be clarified. As such, our current understanding of when and why to operate on retracted TMs is based more on assumptions than on actual evidence.

To investigate the natural history of this poorly understood pathology, a cohort of children with TM retraction was followed over time. All were previously identified during a cross-sectional analysis of a consecutive series of children with cleft palate [3]. This sample was selected because of the higher risk for chronic retraction and sequelae, including hearing loss and cholesteatoma, in the setting of cleft palate [4-7].

OBJECTIVES: The natural history of tympanic membrane retraction is unpredictable. To obtain prognostic information for guiding surveillance and treatment, a cohort of children with retraction from cleft palate were prospectively followed for over 5 years.

MATERIALS and METHODS: This was a prospective observational study at a tertiary academic institution. Children with pars tensa retraction were selected from a cohort of 143 children with cleft palate. Thirty-seven ears were assessed with otoendoscopic image capture and audiometry at a median age of 9 years and reassessed at a median follow-up interval of 6.4 years. The severity of tympanic membrane retraction in the serial images of each ear was compared by four pediatric otolaryngologists blinded to the dates of the images.

RESULTS: Initially, 19/37 retractions (51%) demonstrated contact with the incus and/or promontory. Follow-up images were rated as stable (n=16) or better (n=12) for 28/37 retractions (76%). Of the nine retractions that became more extensive, two developed cholesteatoma (5% of the total). No ossicular erosion developed in ears without cholesteatoma. Conductive hearing loss (4-tone average air-bone gap >25 decibels hearing level) was initially present in five ears, worsened in one, and normalized without intervention in others. No ears with initial normal hearing developed hearing loss.

CONCLUSION: Most tympanic membrane retractions remained stable or improved over time in this cohort of children who were at a risk of persistent eustachian tube dysfunction. Clinically significant progression occurred infrequently, justifying the conservative approach taken to manage these retractions. Such data are necessary to weigh the potential benefit of preventive intervention over observation.

KEYWORDS: Eustachian tube, middle ear, cleft palate, otitis media, chronic otitis media, conductive hearing loss

INTRODUCTION
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This study was presented at the Spring Meeting of the American Society of Pediatric Otolaryngology on May 19, 2017, Austin, Texas, USA.

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ORIGINAL ARTICLE

Natural History of Tympanic Membrane Retraction in Children with Cleft Palate

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MATERIALS and METHODS

Approval for this study was granted by the institution’s Research Ethics Board. Potential study participants were recruited from a cohort of 143 children with cleft palate. All the children had been evaluated with otoendoscopic imaging, audiometry, and tympanometry and were monitored for chronic ear disease according to standard clinical care processes. This typically involved routine 5-year follow-up for normal ears, more frequent observation of retracted TMs according to severity, and operative intervention only for symptomatic middle ear effusions, perforation, or cholesteatoma. All the children in whom retraction of the pars tensa (n=76 ears) had been identified on initial assessment were targeted for prospective follow-up. Most were invited to participate in their routine visits at approximately 5 years after the initial evaluation. Those who had been lost to follow-up (e.g., after graduation to adult services) were contacted and invited to revisit the clinic for evaluation. For patients who could not be reached, data from their last clinic follow-up were used.

Following written consent from the child or caregiver to participate, otoendoscopic imaging was performed by either the senior author or a pediatric otolaryngology fellow. TMs were assessed with 4 or 2.7 mm 0° otoendoscopes, which were positioned and focused to include a view of the entire TM. If necessary, microdebridement was performed to obtain a clear view. Digital images were displayed on a dedicated computer and saved in a secure networked drive. During the same visit, pure tone audiometry and tympanometry were completed. An interval history was also obtained, and any otologic surgery after the initial assessment was noted. For patients who underwent otologic surgery, images and audiometric data from the clinic visit just prior to the surgery were used in the analysis to ensure that no surgical intervention occurred between the visits during which the images were captured.

Image pairs were later created for each retracted TM, with one image extracted from the initial study and second from the reassessment. A study number was assigned to each pair, and all the identifying information was removed. The image pairs were then compiled into a PowerPoint (Microsoft, Redmond, WA, USA) presentation for review. The order of the side-by-side images (initial vs. follow-up, follow-up vs. initial) was randomized. The PowerPoint presentation was assessed on two separate occasions (>1 week apart) by four pediatric otolaryngologists, all of whom had a subspecialty focus in otology. Each reviewer was blinded to the order of the images. Acknowledging that the application of traditional staging systems to TM image review has poor reliability, reviewers were not asked to stage the retractions. Instead, they were instructed to identify which pars tensa appeared worse overall and which appeared worse with respect to each of the following clinically relevant variables: 1) size (surface area of the retraction), 2) visibility of edges and depth, 3) contact with promontory, 4) contact with incus or stapes or both, 5) ossicular erosion, and 6) cleanliness. If there was no appreciable difference or any of the aforementioned variables was not present in any image, then reviewers could select “no difference.”

The primary outcome measure was evaluated based on whether the pars tensa became more or less retracted overall. For data analysis, the study code was broken to determine whether retractions were graded as worse, better, or unchanged over time. The majority of assessments across the four reviewers were used to determine the overall change in appearance. In the event of a 2-2 split, the retraction was considered stable. To facilitate statistical analysis, retractions rated as better or stable were grouped together. This grouping was justified by the lack of a distinction between the two assessments with respect to clinical decision-making. Intra- and inter-rater Kappa statistics with 95% confidence intervals were calculated. Using the criteria described by Landis and Koch, Kappa statistics were then interpreted for reliability.

To determine the incidence of cholesteatoma from TM retraction during the study period, an institutional pathology database was cross-referenced to identify all diagnoses of cholesteatoma arising within the initial cohort of 76 retracted TMs.

Secondary analyses were performed to look for features associated with deterioration (worsening appearance or hearing) at the time of initial assessment. Worsening of hearing was defined as an elevation of the pure tone average (PTA) of >5 dB. The senior author reviewed the initial images on two separate occasions and assessed for the presence or absence of the following: promontory contact, incus contact, stapes contact, depth of retraction out of sight, and accumulation of keratin medial to the annulus. During this review, retractions were dichotomized into two groups: mild retraction not contacting the middle ear structures (equivalent to Sade stage 1) and more severe retraction (contact with the incus or promontory or both, i.e., Sade stage >1). The Fisher’s exact test was employed to investigate whether each variable was associated with a change in appearance or hearing over time. Initial PTAs were compared between the group of retractions that worsened and the group that remained stable or improved using a pooled t-test. Initial PTAs were also compared between the group of retractions that developed further hearing loss and that with stable or improved hearing using a t-test with Satterthwaite adjustment (unequal variances).

Additional secondary analyses were performed to determine whether deterioration of any individual variables assessed was associated with the deterioration of the overall appearance or hearing. Again, the Fisher’s exact test was used for analyzing categorical data, and t-tests were employed for evaluating the association with PTA at follow-up.

Statistical Analysis System Version 9.4 (SAS Institute, Cary, NC, USA) was used for all statistical analyses, which were performed by an independent biostatistician.

RESULTS

Of the 76 pars tensa retractions identified in the original study, 37 (15 unilateral, 11 bilateral) were re-evaluated in 26 patients at a median follow-up interval of 6.4 years (range, 0.75-7.6 years). The median age at reassessment was 15 years (range, 9-21 years). Fifteen patients had a history of complete hard palate cleft (seven with associated cleft lip), seven had incomplete clefts of the hard palate, and four had a history of soft palate cleft alone. Two children were syndromic (Stickler, van der Woude) and three were born with Pierre Robin sequence.
Natural History of Retractions
Follow-up images were rated as stable (n=16) or better (n=12) for 28/37 retractions (76%). No obvious ossicular erosion developed during the study period. Of the nine retractions that worsened, two (5%) ultimately required surgery for cholesteatoma during the study period (diagnosed at 17 and 44 months after the original assessment). No additional operative interventions occurred during the study period. Conductive hearing loss (4-tone average air-bone gap >25 dB-HL), initially present in five ears with retraction, worsened in one ear (PTA elevation from 28 to 50 dB) and normalized without intervention in others. No ears with initially normal hearing developed hearing loss.

The institutional pathology database used to determine this prevalence was also cross-referenced with the entire list of patients examined during the previous study (n=76 retracted TMs), and no other instances of cholesteatoma were identified. Original images were saved between January 2007 and June 2008, and the pathology database was queried in December 2016. Thus, the incidence of cholesteatoma in our original cohort of children with cleft palate with TM retraction over an 8.5-year period was 2.6%.

Reliability of Retraction Assessment
Intra-rater agreement ranged from moderate to 100% across all comparisons. For the primary outcome measure comparing the overall change in severity between the two otoscopic images, inter-rater agreement was moderate. With respect to the individual variables, assessments of bony contact (promontory or incudostapedial) and cleanliness proved to be reliable (moderate to substantial agreement). There was more variability in comparing the depths of the retraction pockets (fair agreement) and ossicular erosion (slight agreement).

Lack of Association between Individual Features and Deterioration
Features noted on unblinded review of the initial images are detailed in Table 1, along with initial tympanometric data. The only feature initially present in both ears that later developed into cholesteatoma was promontory contact. Regarding the whole cohort, however, 88% (14/16) of retractions with promontory contact remained free of cholesteatoma over the study period. Based on these data, the positive predictive value of promontory contact leading to cholesteatoma formation was only 0.125 (95% confidence interval, 0.025-0.125). Neither retraction that led to cholesteatoma demonstrated contact with the ossicular chain, incomplete visibility, or accumulation of keratin debris at the initial assessment.

There was no association between initial retraction severity (mild/Sade 1 vs. more severe/Sade >1) and worsening appearance (p=0.71) or hearing (p=0.60) according to the two-tailed Fisher’s exact test. There were no significant associations between the presence of clinically relevant features assessed (promontory contact, incus contact, stapes contact, depth of retraction out of sight, and accumulation of keratin medial to the annulus) and deterioration of the overall tensa appearance or hearing.

The mean initial PTA for retractions that worsened at follow-up was 10 dB, while the mean for retractions that improved was 17 dB; this difference was not significant (p=0.11, pooled t-test). The mean initial PTA for retractions that developed elevated pure tone thresholds over the study period was 24 dB, whereas the mean for retractions with stable or improved hearing was 11.21 dB; this difference was not statistically significant (p=0.275, t-test with Satterthwaite correction).

Characteristics of Deterioration
Worsened appearance with respect to both promontory contact (19%) and incudostapedial contact (8%) was significantly associated with overall worsening of the retraction (p<0.01 and p=0.01, respectively, two-tailed Fisher’s exact test). There were no significant associations between any variables that worsened and hearing loss (Table 2).

DISCUSSION
In this study sample of children at risk for persistent eustachian tube dysfunction from cleft palate, most TM retractions remained stable or improved over time. They were managed conservatively in this series, thereby allowing for observation of the natural history of the pathology. Clinically significant progression occurred infrequently, at least during the time course followed in this study, which approximates to the teenage years. The overall incidence of cholesteatoma development in children with cleft palate with TM retraction was 2.6% over the 8.5-year period, which was predominantly during the teenage years. This is comparable with the 2% risk of acquired cholesteatoma in all children with cleft palate reported from our center previously (6). In our healthcare system, it is improbable that children from this series would have undergone surgery for cholesteatoma at another institution. None of the other ears followed in this series demonstrated any visible sign of ossicular erosion, and hearing thresholds deteriorated in only one ear. No ears with initially normal hearing thresholds developed hearing loss, although one diffusely

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Table 1. Characteristics of initial retractions noted on unblinded review

<table>
<thead>
<tr>
<th>Feature</th>
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</thead>
<tbody>
<tr>
<td><strong>Tympanometry</strong></td>
<td></td>
</tr>
<tr>
<td>Type A</td>
<td>15</td>
</tr>
<tr>
<td>Type B</td>
<td>8</td>
</tr>
<tr>
<td>Type C</td>
<td>9</td>
</tr>
<tr>
<td>Not tested</td>
<td>5</td>
</tr>
<tr>
<td><strong>Severity</strong></td>
<td></td>
</tr>
<tr>
<td>Mild (shallow, Sade 1)</td>
<td>18</td>
</tr>
<tr>
<td>More severe (Sade &gt;-1)</td>
<td>19</td>
</tr>
<tr>
<td>Promontory contact</td>
<td>16</td>
</tr>
<tr>
<td>Incus contact</td>
<td>7</td>
</tr>
<tr>
<td>Stapes contact</td>
<td>5</td>
</tr>
<tr>
<td><strong>Visibility</strong></td>
<td></td>
</tr>
<tr>
<td>Depth in view</td>
<td>30</td>
</tr>
<tr>
<td>Depth of out of sight</td>
<td>7</td>
</tr>
<tr>
<td><strong>Cleanliness</strong></td>
<td></td>
</tr>
<tr>
<td>No debris</td>
<td>33</td>
</tr>
<tr>
<td>Keratin medial to annulus</td>
<td>4</td>
</tr>
</tbody>
</table>

retracted TM with both promontory and incus contacts demonstrated progression of hearing loss.

Based on the finding that only 1 in 50 children with cleft palate develop cholesteatoma throughout adolescence \[6\], this study did not have the power to definitively ascertain the risk of cholesteatoma formation from TM retraction in this population. Therefore, the observations should not be dismissed because there is a paucity of longitudinal data pertaining to TM retractions. The relatively small sample size of this study is simply a reflection of the difficulty in following a cohort in this age range prospectively over a 5-year period. Although only 37 out of 76 retractions identified in the pilot study were captured in the follow-up, the remaining 38 were cross-referenced with an institutional pathology database, and no additional cases of cholesteatoma were identified.

The objective of this study was to provide data on the natural history of pars tensa retraction, which could be used to compare the effectiveness of outcomes from surgical treatment. For example, from our findings, it can be estimated that to prevent cholesteatoma in teenagers with cleft palate and a pars tensa retraction contacting the promontory or incus, the number needed to treat would be nine (assuming that the intervention is effective 100% of the time). If the intervention had to be repeated annually, similar to that in repeated tympanostomy tube insertion used for management, approximately 80 interventions may be necessary to prevent one case of cholesteatoma from a retraction with bony contact. These are approximate estimates because treatment outcome is unlikely to be 100% effective, and unintended consequences of intervention, such as retained tympanostomy tubes or subsequent TM perforation, may alter the outcome. Furthermore, many other factors must be evaluated when considering the appropriateness of surgical intervention. These include, but are not limited to, the progression of retraction and development of adherence, presence of otitis media, hearing thresholds, status of the contralateral ear, and parental or patient preference. Nevertheless, this series provides some necessary understanding of the natural history of the disorder that can be incorporated into clinical decision-making when surgical intervention is being considered.

More than 50% of TM retractions followed in this study were noted to have bony contact (i.e., Sade stage >1) at the original assessment. A priori, we had hypothesized that this subgroup would be more likely to progress than that with Sade stage 1 retractions. At the end of the study, no group showed much progression and the difference between the two groups was not statistically significant. In fact, none of the factors examined were associated with worsened appearance or hearing over time. Similarly, there was no association between initial PTA and natural history. Taken together, our study was simply underpowered to uncover any factors associated with deterioration.

Recognizing that the progression or improvement of TM retractions is not necessarily ordinal when using traditional staging systems, we believed that serial endoscopic image comparison would more effectively capture the natural history of this disease entity. The factors included in the image review were thought to be clinically relevant and were extrapolated from a multicomponent assessment previously shown to have substantial inter-rater reliability \[3\]. In this study, reviewers were asked to consider all individual factors before making an overall assessment of the pars tensa. Inter-rater agreement for this primary outcome measure was ultimately moderate. However, the intra-rater agreement was better (moderate for one reviewer, substantial for two, and almost perfect for another). This discrepancy may be reflective of differences in the relative significance ascribed to each feature across clinicians and highlights one of the challenges facing clinicians making decisions about how to manage TM retraction.

There are some notable limitations to evaluating the natural history of retraction with endoscopic image comparison. First, given the static nature of the image, adherence cannot be adequately assessed. Although adherence is often mentioned as an important characteristic of TM retraction severity, there is a scarcity of studies validating the assessment with different techniques (i.e., Valsalva maneuver, pneumatic otoscopy, nitrous oxide anesthesia, or surgical dissection). Additionally, there is a lack of evidence with respect to any correlation between adherence and prognosis. It has also been stated that ossicular adherence is irreversible \[10, 11\]. As such, some authors advocate prophylactic tympanoplasty when ossicular contact is identified to prevent the erosion of the incus \[12\].

Table 2. Lack of association between initial hearing thresholds and deterioration of retraction features

<table>
<thead>
<tr>
<th>Variable (n from majority assessment)</th>
<th>Mean PTA in dB</th>
<th>t-test p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worse (7)</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>Better (30)</td>
<td>13.0</td>
<td>0.93</td>
</tr>
<tr>
<td>Visibility of depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worse (2)</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Better (35)</td>
<td>12.9</td>
<td>0.98</td>
</tr>
<tr>
<td>Promontory contact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worse (7)</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Better (30)</td>
<td>13.0</td>
<td>0.88</td>
</tr>
<tr>
<td>Incudostapedial contact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worse (3)</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>Better (34)</td>
<td>13.2</td>
<td>0.60</td>
</tr>
<tr>
<td>Ossicular erosion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worse (1)</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Better (36)</td>
<td>13.1</td>
<td>0.43</td>
</tr>
<tr>
<td>Cleanliness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worse (5)</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>Better (32)</td>
<td>11.1</td>
<td>0.13*</td>
</tr>
</tbody>
</table>

*Satterthwaite correction applied for unequal variances

PTA: pure tone average

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*Satterthwaite correction applied for unequal variances

PTA: pure tone average
Clinically significant progression of TM retraction was infrequent during the teenage years in our cohort, a group at risk for chronic eustachian tube and middle ear dysfunction from cleft palate. This relatively benign natural history should be considered when weighing the risks and benefits of surgical intervention for TM retraction without cholesteatoma in this age group.

CONCLUSION

Clinically significant progression of TM retraction was infrequent during the teenage years in our cohort, a group at risk for chronic eustachian tube and middle ear dysfunction from cleft palate. This relatively benign natural history should be considered when weighing the risks and benefits of surgical intervention for TM retraction without cholesteatoma in this age group.
Eustachian Tube Function in Adults with Ventilation Tubes Inserted for Otitis Media with Effusion

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OBJECTIVE: To investigate the eustachian tube (ET) function (ETF) in adults with ventilation tube (VT) inserted for the treatment of chronic otitis media with effusion (COME).

MATERIALS and METHODS: A total of 17 subjects with at least one VT were enrolled. A detailed history was obtained, and risk factors were assessed with questionnaires. Examination including nasopharyngeal video endoscopy and ETF tests, the forced response test (FRT), inflation–deflation test (IDT), and nasal/nasopharyngeal maneuvers (such as sniffing and Valsalva, Toynbee, and the diver’s maneuvers) were performed.

RESULTS: Averages for FRT were 580±333 daPa, 382±251 daPa, and 138±192 daPa for opening pressure, steady-state pressure, and closing pressure, respectively. Most subjects demonstrated minimal or weak active function during the FRT and IDT. While nasopharyngeal maneuvers changed the nasal/nasopharyngeal pressures, they did not significantly change the middle-ear pressures. These results indicated that most subjects had severe obstructive ET dysfunction (ETD) with an ET lumen that required high pressure differences to open and poor active muscular function inadequate for luminal dilation. These results imply that while any treatment to widen the ET, such as balloon dilation of the ET, is not expected to change the voluntary active muscular function, it may reduce the tissue pressures and resistance, thus facilitating luminal opening both passively and actively.

CONCLUSION: Most patients with VT inserted for the treatment of COME appear to have an abnormal ETF with difficulty in passively opening the ET and weak active muscular function. Management of such patients addressing only passive properties may not be sufficient for the resolution of ETD.

KEYWORDS: Eustachian tube, eustachian tube dysfunction, middle ear effusion, ventilation tube, balloon dilation of eustachian tube

INTRODUCTION
Optimal hearing requires a healthy middle ear (ME) with an intact tympanic membrane (TM), an intact and mobile ossicular chain, and an aerated ME near ambient pressure. The sum of the gas partial pressures in the ME is higher than that in venous blood; therefore, the gases gradually equilibrate with blood by diffusion[1]. Similar to any sealed gas pocket within the body, unless there is resupply of such gases, ME pressure (MEP) will reach an unsustainable negative value and the ME will either collapse and/or be filled with fluid. The eustachian tube (ET) is the conduit, which resupplies the ME by periodically opening to deliver a bolus of gas into the ME to compensate for the decrease in MEP due to the diffusion of ME gases through the mucosa. An inadequate ET opening frequency and/or gas bolus volume results in a negative MEP[2]. A sustained negative MEP may facilitate ascending viral and bacterial infections and/or lead to fluid transudation or exudation, thus gradually filling the ME.

Eustachian tube dysfunction (ETD) may manifest itself as a chronic condition or a temporary/intermittent problem arising due to external or internal challenges, such as ambient barometric changes or illnesses and conditions, that affect its function[3]. Both intermittent and chronic ETD may be associated with high tissue pressures that increase resistance to opening or weak active opening function or both[4]. A variety of clinical presentations and underlying causes may represent different phenotypes of ETD. Further research is warranted to delineate the clinical and functional characteristics of each presentation within the spectrum of ETD.

This study was presented in part as an oral presentation at the 19th International Symposium on Recent Advances in Otitis Media, 4–8 June 2017, Convention and Exhibition Centre, Gold Coast, Australia.

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Historically, ETD was invoked as the cause of many ME diseases including recurrent otitis media, acute or chronic ME effusion, TM retraction, retraction pockets, cholesteatoma, and medical and surgical treatment failures, even though there was no specific therapy for it. Several targeted treatment methods have recently been introduced, including the relatively non-invasive method of balloon dilation of ET (BDET), which provide hope for a cure for ETD and its sequelae[10]. Despite BDET’s relatively non-invasive nature, little is known regarding its effect on the ET mucosa, the peritubal tissues, and its mechanism of action[6, 7]. A recent controlled study demonstrated the efficacy of BDET in adults with intact TMs and type B or C tympanograms; however, the severity of ETD was not demonstrated with testing in that population[11]. It remains unclear whether BDET is indicated and has a similar benefit for all manifestations and severities of ETD.

Otitis media with effusion (OME) is defined as inflammation of the ME mucosa with the accumulation of effusion in the normally air-filled ME, without the signs and symptoms of acute otitis media. The pathogenesis of ME effusion is multifactorial, with ETD being the most consistently claimed cause both in children and adults[12]. While relatively more common in infants and young children, most grow out of their ME problems, reflected in the decreasing prevalence of ETD and the resulting OME in older children and adults. Only a small proportion continues to have OME into adulthood. Adults who develop chronic OME and require ventilation tube (VT) insertion probably represent the worst ETD manifestation. Therefore, we conducted a study to describe the history, clinical profile, and ET function (ETF) test results in adults who need VT insertion to treat OME; this cohort likely represents the worst ETD manifestation. Therefore, we conducted a study to describe the history, clinical profile, and ET function (ETF) test results in adults who need VT insertion to treat OME; this cohort is assumed to represent patients with ETD for whom BDET may be indicated.

MATERIAL and METHODS

Otherwise healthy male and female subjects aged 18–50 years with unilateral or bilateral VTs inserted for treatment of OME with suspected ETD were enrolled in this study. Subjects were recruited by referral from adult otology clinics and by advertisement. Potential subjects were screened by phone conversation for eligibility and invited for evaluation and testing at Middle Ear and Eustachian Tube Research Center. This study was reviewed and approved by the University of Pittsburgh Institutional Review Board.

After obtaining written informed consent for participation, subjects underwent a full evaluation, including demographic information, a general health history, a specific ear disease history, questionnaires, physical and otologic examination via otomicroscopy and otoendoscopy, nasopharyngeal video endoscopy, and ETF testing.

Questionnaires for ETD, allergies, sinusitis, and gastroesophageal reflux disease (GERD) were completed by the subjects. The Eustachian Tube Dysfunction Questionnaire-7 (ETDQ-7) was used for the evaluation of ET symptoms[10]. The Allergy Control SCORE™ questionnaire was used for the evaluation of allergic rhino-conjunctivitis symptoms[11–13] and the Sino-Nasal Outcome Test-20 (SNOT-20) questionnaire was used for evaluating the presence of rhinosinusitis[14]. The Gastroesophageal Reflux Disease-Health Related Quality of Life (GERD-HRQL) questionnaire was used for the evaluation of reflux. Tymanometry (Titan Middle Ear Analyzer, Interacoustics, Eden Prairie, MN) was performed to confirm the patency of VTs, and in ears without a VT or perforation, the MEP and compliance were recorded. In ears with a patent VT, the volume was recorded. A 0° endoscope (60×2.7 mm, 1215A Karl Storz Endoscopy, Germany) with a pneumatic sleeve (1215Q, Karl Storz Endoscopy, Germany) attached to a high-speed digital camera (uEye 1220SE, IDS Obersulm, Germany) was used to examine the ear canal and TM. Camera signals were continuously split-routed to an online monitor and to the memory of a PC for storage and analysis.

Only one ear of a subject with bilateral non-intact TMs was included for testing and analysis in the study. A panel of five ETF tests was used to assess the study ears with non-intact TMs; these tests were the forced response test (FRT), the inflation–deflation test (IDT), and the maneuvers, including sniffing, Valsalva, and Toynbee. ETF testing was performed with a custom-made instrument developed by us. Components of this instrument include an ear-canal probe serially attached with a tube to an SDX01D4 differential pressure transducer (Honeywell) with a 3-way valve to a flow sensor (Respiratory Flowhead 1L MLT1; AD Instruments) and a second 3-way valve to a variable-speed constant-flow pump (Harvard Apparatus Pump 22; Harvard Apparatus) with a syringe pump controller (Syringe Pump Controller version 1.2; National Instruments); a nasal probe was attached with tubing to an SDX01D4 differential pressure transducer. Signals from the transducer were sent to a PowerLab 16/30 data acquisition system connected to a personal computer running Laboratory Chart software, version 7.3.6 (AD Instruments) that displayed the waveforms and data storage in real time.

An ear-canal probe was sealed into the test ear for the FRT. After opening the valves of the test instrument, an airflow of approximately 11 mL/min was delivered by the constant-flow pump to the ME. This continuous delivery of air increased ME pressure to the level at which the ET lumen was forced to passively open (opening pressure [PO]). As the pump continued to deliver an airflow, the pressure and the flow became stable, resulting in a steady system pressure (PS), where the flow (QS) through the ET was almost equal to the applied flow rate by the pump. An air pump delivered a constant flow through the ear canal, non-intact TM, ME, and the ET, which kept the lumen open. After reaching the steady state, the subject was asked to swallow, which contracts the tensor veli palatini (mTVP) and levator veli palatini (mLVP) muscles. Contraction of these muscles is expected to widen the diameter of ET, usually increasing the airflow through the ET (PA, active pressure, at QA, airflow during a swallow). During the steady-state phase, a swallow with active muscular function is expected to widen the lumen, increase the flow rate, and reduce the resistance at the time of the swallow, which should all go back to the baseline flow rate after the swallow. The absence of widening evidenced by lack of increased flow and decreased resistance and system pressure indicates absent muscle strength. The degree of flow change with the change of the ET lumen is measured as dilatary efficiency (DE), a measurement of how weak or strong the tubal musculature is. At the end of the test session, the pump was turned off, thus resulting in a decrease in airflow and pressure, eventually to a level where the ET passively closed. This measured pressure, expected to be equal to the tissue pressures, was the closing pressure (PC). After completion of the first test sequence, the same testing was repeated at an airflow rate of approximately 23 mL/min. Throughout the test, system pressure and flow were continuously...
recorded. These waveforms were analyzed, PO, PC, PS, QS, and QA were identified and recorded, and three derived parameters were calculated: passive ET resistance (RS=PS/QS), active resistance (RA=PA/QA), and ET DE (DE=RS/RA) for both flow rates. The data were reconciled, and the following parameters were entered into the database for analysis: PO, PC, RS, and DE at each airflow rate. Measures of the passive forces that act to maintain a closed ET lumen are PO and PC; the output of the ease of trans-ET air flow is RS, and RS11/RS23 is considered as the output related to ET compliance. All these parameters are related to the structural or passive properties of the ET. Conversely, the active muscular function is measured with DE, which measures the functional effectiveness in the dilation of the ET lumen with active muscle contraction. 

For the IDT, the test ear was sealed with the ear-canal probe, and after opening the valves, ME pressure was elevated to a pressure of approximately +200 daPa (referenced to ambient pressure), and to reduce system volume, the valves were closed. The subject was then asked to swallow five times at intervals of 3-5 seconds, and the residual pressure (RP) after the 1st and 5th swallow was recorded. MEP was then vented to ambient pressure. The testing was repeated at an applied MEP of approximately –200 daPa. The parameters used in the analysis were the percentage correction of applied ME positive (Infl%Eq) or negative pressure (Def%Eq) after one and five swallows, calculated as applied pressure minus the RP divided by the applied pressure. The ability to open the ET and percentage of equilibration of the positive and negative MEPs were used to evaluate active muscular function, i.e., the efficiency of the muscle-assisted opening of the resting ET lumen.

Continuous measurements of the nasal and ME pressures were obtained during the nasopharyngeal maneuvers. Nasal and ME pressures achieved during the sniffing, Valsalva, and Toynbee maneuvers were recorded.

For the endoscopic examination of the nose and nasopharynx, the nasal passages were topically anesthetized and decongested first with a sprayer and then with a cotton gauge (Medtronic Neura*Neurosurgical Patties, ½ in. x 2 in., Medtronic Xomed, Inc., Jacksonville, FL) comprising a 1:1 solution of 4% lidocaine hydrochloride (Roxane Laboratories Inc., Columbus, OH) and 0.05% Oxymetazoline Hydrochloride (Major* Soothing 12 Hour Nasal Decongestant Spray, Major Pharmaceuticals, Livonia, MI). A 0° endoscope (Hopkins, 2.7 mm. 18 cm, Karl Storz Endoscopy, Germany) attached to a high-speed digital camera was used to examine the nasal passages. The endoscope was then advanced through the experimental side (had the VT and ETF testing) to the level of the nasopharynx. The endoscope was removed; a 45° telescope (Hopkins, 2.7 mm. 18 cm, Karl Storz Endoscopy, Germany) with the attached video camera was inserted into the same side, advanced to the nasopharynx, and focused on the ipsilateral ET orifice. Movements of the ipsilateral ET and associated structures were visualized and captured while the subject performed three sequential swallows. The state of soft palate elevation, degree of rotation of the posterior lamina of the ET cartilage, and degree of widening of ET orifice was assessed at baseline (T1), at the time of maximum elevation of the soft palate during swallow (T2), at the time of maximum widening of the ET orifice (T3), and at the end of swallow (T4).

**Statistical Analysis**

In the current manuscript, due to small sample size, Excel (2016 for Windows, Microsoft, Redmond, Washington, USA) Data Analysis tool “Descriptive Statistics” was used. The test results and findings were compared to previously reported values of healthy adults with and without a history of ETD.

**RESULTS**

A total of 17 subjects; 11 males and 6 females, all non-Hispanic white, with an average age 31.3±10.2 (range 18.5–45.6) years were enrolled. Thirteen subjects had unilateral VTs (7 right and 6 left), three had bilateral tubes, and one had unilateral (right) perforation after a recent extrusion of a VT. Test results of only one ear per subject were included in the analysis. For subjects with bilateral VTs, test results of the worse ear were included.

Subjects were otherwise healthy, with chronic and/or recurrent middle-ear problems, especially when they did not have VTs. However, some subjects expressed their symptoms and complaints based on their recall because they experienced those during the usually short interval when a tube had extruded until the new one was inserted. Daily pressure in the ears was present in 10 (58.8%), 4 (23.5%) felt popping, and 2 (11.8%) subjects expressed pain; 11 (64.7%) felt symptoms in the test ear with altitude change, 15 (88.2%) with colds. One subject engaged in scuba diving and felt pressure and popping during this activity. All subjects had experience flying; 12 (70.6%) became symptomatic during or after a flight, 5 (29.4%) felt fullness and pressure, 4 (23.5%) popping, and 2 (11.8%) pain. To alleviate the discomfort, 6 (35.3%) performed Valsalva, 6 (35.3%) yawned, 3 (17.6%) moved their jaw, and 3 (17.6%) chewed gum; 5 (29.4%) stated that nothing helped. Ten (58.8%) subjects stated that they heard their own voice and 8 (47.1%) heard their breathing at least sometimes.

The majority of subjects had ear infections since they were a child. During childhood, 14 (82.4%) subjects recalled having an earache, 13 (76.5%) having ear infections or fluid, 15 (88.2%) receiving antibiotics, and 14 (82.4%) having VTs inserted. All subjects experienced ear infections or fluid as adults and received VTs. In the test ear, subjects recalled having had 8.6±5.5 sets of tubes (range from 2 to ≥23). The last set of tubes was placed at an average age of 29.9±10.5 years (range 17–45). A history of hearing loss was recalled by 13 (76.5%), dizziness by 5 (29.4%), ringing in the ear by 10 (58.8%), and perforation of the TM in the test ear by 5 (29.4%). Past surgical history included adenoidectomy in 8 (47.1%), tonsillectomy in 7 (41.2%), tympanoplasty in 4 (23.5%), and ossicular chain reconstruction in 1 subject (5.9%). A family history of ear infections or fluid and VT insertion was present in 6 (35.3%).

Review of systems revealed a history of nasal allergies in 10 (58.8%) and asthma in 4 (23.5%) subjects. Sixty-five percent of subjects reported having been tested for allergies, 9 (52.9%) reported seasonal allergies, and 3 (17.6%) reported food allergies. Associated symptoms reported by subjects are listed in Table 1. Six (35.3%) were on antihistamines, and 1 subject (5.9%) was on nasal topical steroids. Although 9 (52.9%) reported some symptoms associated with gastroesophageal reflux disease (GERD), only 2 (11.8%) regularly took reflux medications (1 only proton pump inhibitor, 1 both proton pump inhibitor and H2 blocker), and 1 (5.9%) used antacid as needed. High blood pressure was present in 1 subject (5.9%), cough in 3 (17.6),
nervousness in 3 (17.6%), and sleep problems in 4 (23.5%). There was a smoking history in 6 (35.3%) subjects (duration: 3–22 years); 4 (23.5%) reported having quit smoking. Four subjects (24%) reported a recent weight loss.

The mean ETDQ-7 score was 3.4±1.4 (range 1–5.9); 13 (76.5%) were greater than an average score of 2.5 of the total average score of 7, indicating that they experienced moderate to severe ETD problems (Table 2). Responses to the GERD-HRQL questionnaire indicated normal scores for the heartburn indicators in 11 (64.7%), for regurgitation indicators in 15 (88.2%), and QOL indicators in 11 (64.7%). Average total score of the Allergy Control SCORE was 10.9±10.4 (range 0–34). Average total SNOT-20 questionnaire score was 16.4±13.4 (range 0–43), and the mean score was 0.8±0.7 (range 0–2.2).

Among the continuous variable ET function tests (Table 3), the FRT at 11 mL/min pump speed revealed an average opening pressure of 580±333 mmH2O (range 201–1363). The average closing pressure was 138±192 mmH2O (range 14–755). The average opening pressure and closing pressure on FRT at 23 mL/min were 501±271 mmH2O (range 196–1023) and 109±156 (range 0–653), respectively. The ratio of steady-state resistance of two pressures (RS 11/RS 23), a measure of compliance calculated in 15 subjects (88.2%) was 2.2±0.8 (range 1–4). The average percent equilibration of the pressure difference between the 1st and total of 5 swallows in IDT for the inflation test (Infl%Eq; when ME pressure is high compared to ambient) were 14%±18.9% and 33.8%±29.2% respectively. For the deflation test phase (when ME pressure is low compared to ambient) the average percent correction for the 1st and 5th swallows (Def%Eq) were 7.5%±9.9% and 22.4%±22%, respectively (Table 4).

Pressure measurements of the nose and the ear canal (equal to the ME with the VT) obtained during the nasopharyngeal maneuvers, including the sniffing, Valsalva, and Toynbee, are summarized in Table 5. Sniffing and Valsalva maneuvers were performed in 13 (76.5%) and Toynbee was performed in 12 (70.6%) subjects. With the sniffing maneuver, only one ear pressure changed 33% of the nasal pressure, two ears changed 5% and 3%, respectively, while the remaining showed no changes in ear pressure at all. Three of the six subjects that exceeded nasal pressures of 600 daPa during Valsalva, raised their ME pressure to 77%, 93%, and 98% of the nasal pressure. The other three with nasal pressures reaching 646, 670, and 1054 daPa increased their ME pressures by 0%, 1%, and 0% of those values, respectively. While half of the nasal pressures decreased with Toynbee, the other half either did not change much or increased. The changes in ME pressure with the Toynbee maneuver were minimal.

Table 1. Symptoms potentially associated with nasal allergies reported by subjects (n=17)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>At present</th>
<th>In the past</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Stuffy nose</td>
<td>6</td>
<td>35.3</td>
</tr>
<tr>
<td>Sneezing</td>
<td>5</td>
<td>29.4</td>
</tr>
<tr>
<td>Itchy eyes</td>
<td>4</td>
<td>23.5</td>
</tr>
<tr>
<td>Runny nose</td>
<td>3</td>
<td>17.6</td>
</tr>
<tr>
<td>Frequent colds</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>Mouth breathing</td>
<td>6</td>
<td>35.3</td>
</tr>
<tr>
<td>Snoring</td>
<td>7</td>
<td>41.2</td>
</tr>
</tbody>
</table>

n: number

Table 2. Eustachian Tube Dysfunction Questionnaire (ETDQ-7) results (n=17)

<table>
<thead>
<tr>
<th>SYMPTOMS</th>
<th>avg</th>
<th>std. dev</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure in the ears?</td>
<td>3.9</td>
<td>1.8</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Pain in the ears?</td>
<td>2.9</td>
<td>1.8</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>A feeling that ears are clogged or “under water?”</td>
<td>3.8</td>
<td>2.2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Ear symptoms when you have a cold or sinusitis?</td>
<td>3.0</td>
<td>1.8</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Cracking or popping sounds in the ear?</td>
<td>3.6</td>
<td>1.7</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Ringing in the ear?</td>
<td>2.8</td>
<td>1.8</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Muffled sound?</td>
<td>3.6</td>
<td>1.9</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Mean score</td>
<td>3.4</td>
<td>1.4</td>
<td>1</td>
<td>5.9</td>
</tr>
</tbody>
</table>

avg: average; std. dev: standard deviation; min: minimum; max: maximum

Table 3. Forced Response Test (FRT) results for continuous variables

<table>
<thead>
<tr>
<th>FORCED RESPONSE TEST (FRT)</th>
<th>Pump rate at 11 mL/min</th>
<th>Pump rate at 23 mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>avg</td>
</tr>
<tr>
<td>Opening pressure (PO)</td>
<td>16</td>
<td>580</td>
</tr>
<tr>
<td>Steady-state pressure (PS)</td>
<td>15</td>
<td>382</td>
</tr>
<tr>
<td>Steady-state flow (QS)</td>
<td>15</td>
<td>11.3</td>
</tr>
<tr>
<td>Steady-state resistance (RS)</td>
<td>15</td>
<td>34</td>
</tr>
<tr>
<td>Swallow pressure (PA)</td>
<td>15</td>
<td>380</td>
</tr>
<tr>
<td>Swallow flow (QA)</td>
<td>15</td>
<td>32</td>
</tr>
<tr>
<td>Swallow resistance (RA)</td>
<td>15</td>
<td>131</td>
</tr>
<tr>
<td>Closing pressure (PC)</td>
<td>15</td>
<td>138</td>
</tr>
<tr>
<td>Dilatory efficiency (DE)</td>
<td>15</td>
<td>3.2</td>
</tr>
</tbody>
</table>

n: number; avg: average; std. dev: standard deviation; min: minimum; max: maximum
Video recordings of the endoscopic examination of the nasopharynx with the 0° and 45° endoscopes were available for analysis in 15 (88.2%) subjects (two subjects refused the nasal endoscopy after ETF testing). Those documented a mild, moderate, or large degree of lymphoid tissue in the nasopharynx in 11 (73.3%), in the fossae of Rosenmüller in 13 (86.7%), over the ET cartilage in 6 (40%), and within the ET lumen in 6 (40%) of subjects (Table 6). Inflamed mucosa on the torus tubarius was present in 8 (53.3%). Abundant secretions were present in the nasopharynx and over the ET orifice in 13 (86.7%) and 10 (66.7%) subjects, respectively. Frame-by-frame analysis of videos taken during two swallows showed the average total duration of swallows was 3.42±1.55 s, while time to a maximum elevation of the soft palate was a 1.42±0.86 s, with maximum widening of the ET orifice occurring 0.47±0.37 s after the maximum palatal elevation. Mild constriction of the ET orifice during soft palate elevation was seen in 10% of swallows.

Pearson correlation analysis performed on all the quantitative and semi-quantitative variables is summarized in Table 7. Because the full set of associations is impossible to include, variables that have a higher number of significant associations are selected for presentation. Among the questionnaires, SNOT-20 and of the ETF test parameters, DE had the highest number of significant associations with other parameters. However, overall, the low number of significant associations suggest a high degree of variability among the subjects, and may be due to the small number of subjects in the study. It is also possible that conditions the questionnaires assess may not be associated with ETF and ME disease in subjects with a long history of ETD.

DISCUSSION

Previously, ETD was a presumptive diagnosis assigned, without any testing, to ears with symptoms of pressure pain, an uncomfortable degree of popping, an inability to tolerate pressure variations resulting in middle-ear effusion, or hemotympanum and to patients diagnosed with recurrent otitis media, acute or chronic ME effusion, TM retraction, retraction pockets, cholesteatoma, and medical and surgical treatment failures. Most practitioners do not use the various published tests to verify this diagnosis because results do not guide the management of ETD or the resulting clinical condition. Moreover, the fact that these testing methods are not readily available or easily performed, their sensitivity and specificity are not fully documented, and their interpretation requires expertise, compounds this situation.

This situation changed after the discovery of a non-invasive presumably effective treatment, the BDET[5, 15]. Clinicians then felt it challenging to identify the correct indications for this procedure and assess and document its effects. Naturally, reimbursement and medicolegal concerns were among the main driving forces in seeking clarity with respect to defining abnormal ETF. In the absence of the broadly utilized accurate test methods, elements of history, symptoms, questionnaires for symptom scores, and ability to perform Valsalva were used as substitutes for the tests[16]. Tympanometry and tubomanometry results were also added to the ETD test batteries [17, 18] creating scoring systems that go from a simple questionnaire (ETDQ-7) to one that requires a test machine (Tubomanometer) and is only available in a few centers (ETS-7). Recognizing the limitations of these diagnostic criteria, an alternative approach was proposed by the manufacturers of the recently approved balloon dilation device: evidence of negative ME pressure and endoscopic verification of inflammation on and around the ET orifice were added to abnormal ETDQ-7 scores.
for indication to perform balloon dilation. This approach was based on the claim that the role of balloon dilation is not via the structural changes it causes but via the elimination of the mucosal inflammatory changes at the orifice and in the lumen.\(^7\) Irrespective of the mechanism of action, there is an ongoing need for more sensitive and specific methods for measuring ETF.

In this study, all 17 ears had abnormal ETF as per the previously reported values and ranges of normal and abnormal test results\(^14\). Of these 17 ears, all had an abnormality in the ET pressure-equalizing function. Fourteen (82.4%) had abnormal passive function properties, i.e., very high-pressure differences between the ME and ambient were needed to passively open the ET. The active function was abnormal in 16 (94.1%), i.e., even when there was a sufficient ME-nasopharynx pressure gradient, the muscle activity was unable to sufficiently widen the ET to equilibrate the pressure difference. Thirteen (76.5%) ears with both abnormal passive and active functions were categorized as having severe ventilatory dysfunction. Three (17.6%) ears with normal passive but abnormal active properties were assigned as having moderate ventilatory dysfunction. The only subject with abnormal passive but normal active function was considered to have mildly abnormal ventilatory ET function.

The protective function of the ET was assessed through the opening and closing pressures. None of the subjects had low opening pressure; however, 7 ears (41.2%) had a low closing pressure, indicating an abnormality in the protective function. Six of the seven ears with abnormal protective function had a severely abnormal pressure-equalizing function, indicating that although those ETs had difficulty in opening passively and actively, once opened, they closed at low pressures, making them prone to regurgitation of nasopharyngeal secretions possibly containing the virus and/or bacteria.

<table>
<thead>
<tr>
<th>Selected Pearson Correlation Analysis Results</th>
<th>Questionnaire Scores</th>
<th>Video Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Video Analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degree of ET cartilage rotation at T2</td>
<td>Slope: −0.61</td>
<td>p: 0.026*</td>
</tr>
<tr>
<td></td>
<td>Score: 0.07</td>
<td>n: 13</td>
</tr>
<tr>
<td></td>
<td>−0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−0.17</td>
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</tr>
<tr>
<td></td>
<td>−0.41</td>
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</tr>
<tr>
<td></td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Degree of lumen opening at T2</td>
<td>Slope: 0.04</td>
<td>p: 0.89</td>
</tr>
<tr>
<td></td>
<td>Score: −0.18</td>
<td>n: 13</td>
</tr>
<tr>
<td></td>
<td>−0.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−0.33</td>
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</tr>
<tr>
<td></td>
<td>−0.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Degree of soft palate relaxation at T3</td>
<td>Slope: −0.13</td>
<td>p: 0.66</td>
</tr>
<tr>
<td></td>
<td>Score: 0.06</td>
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<td></td>
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<tr>
<td></td>
<td>−0.08</td>
<td></td>
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<tr>
<td>Degree of constriction at T3</td>
<td>Slope: −0.18</td>
<td>p: 0.56</td>
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<td></td>
<td>Score: 0.05</td>
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<tr>
<td></td>
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</tr>
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</tr>
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<td></td>
<td>0.67</td>
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<tr>
<td></td>
<td>0.06</td>
<td></td>
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<tr>
<td></td>
<td>0.02*</td>
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<tr>
<td>ETF Test Results</td>
<td>Slope: 0.50</td>
<td>p: 0.06</td>
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<tr>
<td>Steady-state resistance at 11 mL/min</td>
<td>Score: 0.28</td>
<td>n: 15</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.54</td>
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<td></td>
<td>0.57</td>
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<tr>
<td></td>
<td>−0.53</td>
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<tr>
<td></td>
<td>−0.10</td>
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<tr>
<td>Dilatory efficiency at 11 mL/min</td>
<td>Slope: 0.65</td>
<td>p: 0.008*</td>
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<tr>
<td></td>
<td>Score: 0.44</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>−0.11</td>
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<tr>
<td>Opening pressure at 23 mL/min</td>
<td>Slope: 0.42</td>
<td>p: 0.10</td>
</tr>
<tr>
<td></td>
<td>Score: 0.22</td>
<td>n: 17</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
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<tr>
<td></td>
<td>0.50</td>
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<td>−0.61</td>
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<td></td>
<td>−0.02</td>
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<tr>
<td>Inflation % equilibration at 1st swallow</td>
<td>Slope: 0.22</td>
<td>p: 0.39</td>
</tr>
<tr>
<td></td>
<td>Score: 0.49</td>
<td>n: 17</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>0.69</td>
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</tr>
</tbody>
</table>

ETF: Eustachian tube dysfunction questionnaire; GERD: gastroesophageal reflux disease; SNOT: sino-nasal outcome test; ETF: Eustachian tube function

*Correlation coefficients with \(p<0.05\); T2: time of maximum elevation of soft palate during swallow; T3: time of maximum widening of the ET orifice
Questionnaires to identify the presence of risk factors for OM and ETD in the study population, such as nasal allergy, sino-nasal symptoms, and GERD, were used to identify potential risk factors for inflammation in and around the ET. However, despite reports in the literature suggestive of these associations, only a few subjects in this study appeared to have relatively high scores.

Current evidence suggests that there may be different ETD phenotypes and that a testing algorithm that produces relevant discriminations is necessary to identify a specific phenotype. Our Center has developed a number of tests, including the FRT and IDT, applicable to ears with a VT or a perforation \cite{39}. In our recent publication, we compared ETF tests between 15 normal ears of 15 adult subjects who received experimental myringotomy with 23 ears of 19 subjects with ventilation tubes inserted for ETD \cite{40}. This study demonstrated that the 4 ETF test parameters (Valsalva, ET opening pressure, DE, and percentage of positive pressure equilibrated) together correctly identified ears with ET dysfunction with a sensitivity of 95% and a specificity of 83%. The 19 subjects in that study had unilateral or bilateral VTs inserted by their physician for the diagnosis of ETD. However, only 20 of the 23 ears had a history of COME, and of the 20, 10 also had a history of acute and/or recurrent acute otitis media, representing a variability in the ETD profile. Conversely, the current study was conducted on a separate more uniform group, defined by having VT insertion for COME, as a distinct group with worse anticipated ETD, not reported previously. The average opening pressure on the FRT in this study was much higher (580±333 daPa at 11 mL/min and 501±271 daPa at 23 mL/min) compared with both study and control groups in the previous publication (approximately 300 daPa for both groups, both pump speeds). Similarly, closing pressures were higher for a similar comparison. These parameters represent a much higher tissue pressure resistance to open and close the ET lumen in the ears with a more severe ETD. Dilatory efficiencies of 3.2±4.1 and 1.2±1.5 for the 11 and 23 mL/min pump speeds, respectively, in the current study, were not different from the results of the ETD group (3.2±3.1 and 2.5±2.2 for 11 and 23 mL/min, respectively) in the previous study. However, DE in the control group from the previous publication was higher (8.7±7.4 and 4.5±2.8 for 11 and 23 mL/min, respectively) compared to the test results of both ETD groups. DE, the measure of active muscular dilation that widens the ET lumen is apparently abnormal in all forms of ETD. The difference between the current study group and the ETD group in the previous publication was more apparent in the inflation–deflation test results. The percent equilibration of positive pressure and negative pressure after 5 swallows were 33.8±29.2% and 22.4±22% for this study versus 57±36% and 33±38% for the ETD group, respectively. This suggests that ETD was more severe in this population, with its more stringent enrollment criteria, VTs for the indication of COME as an adult, compared with the more diverse cohort reported previously. The current study population with a history of VT insertion as a child in 82%, and an average of 8.6±5.5 sets of tubes (range from 2 to >23) is also clearly different from the study that served to obtain FDA approval for the balloon dilation device on adults with no history of prior VT insertion 39.4%, and only one set of VT in 41.3%, implying very small overlaps between the two study populations \cite{41}.

Of these tests, it appears that abnormal passive test results from ETF (opening and closing pressures) are more consistently associated with more severe forms of ETD, similar to those ears with persistent effusion when a VT is not inserted. Of the active function tests, DE is abnormal in most forms of ETD, and the ability to equilibrate positive and negative pressures is worse in ears with VT insertion for chronic effusion.

Results of this study indicate that most subjects had severe obstructive form to ETD, with an ET lumen that required high-pressure differences to open and poor active muscular function inadequate for luminal dilation. These results imply that while any treatment to widen the ET, such as balloon dilation of ET, is not expected to change the voluntary active muscular function, it may reduce the tissue pressures and resistance, thus facilitating luminal opening both passively and actively.

**CONCLUSION**

Currently, to the best of our knowledge, no test is widely available, easy to use, objective, and reproducible for testing ET function. Sophisticated test methods are still under investigation with respect to their sensitivity and specificity and are used in few centers of the world. Lack of objective and reliable tests leads to the use of surrogate and mostly subjective criteria for diagnosing ETD and determining the best treatment method. The current study suggests that the presence of VTs in adulthood inserted in response to the development of ME effusion indicates severe ETD, and most patients appear to have abnormal ETF with difficulty passively opening the ET and with a weak active muscular function. An effort should be made to address the extrinsic contributors to ETD, such as inflammation, adenoid, and lymphoid tissue, prior to treatment of the ET itself. However, management of such patients addressing only passive properties may not be sufficient for the resolution of ETD. Therefore, such patients with relatively worse ETD should be warned that the severity of their dysfunction may limit the benefit of the available treatment methods.

**Ethics Committee Approval:** Ethics committee approval was received for this study from University of Pittsburgh Institutional Review Board.

**Informed Consent:** Written informed consent was obtained from the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - C.M.A.; Design - C.M.A.; Supervision - C.M.A., M.S.T., J.D.S.; Resource - C.M.A.; Data Collection and/or Processing - C.M.A., M.S.T., J.D.S.; Analysis and/or Interpretation - C.M.A., M.S.T., J.D.S.; Literature Search - C.M.A.; Writing - C.M.A.; Critical Reviews - C.M.A., M.S.T., J.D.S.

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**REFERENCES**


Using Non-Echoplanar Diffusion Weighted MRI in Detecting Cholesteatoma Following Canal Wall Down Mastoidectomy – Our Experience with 20 Patient Episodes

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OBJECTIVE: To our knowledge, there is no study exploring specifically the diagnostic performance of diffusion-weighted imaging (DWI) in patients with previous canal wall down (CWD) surgery when combined with appropriate clinical evaluation. The aim of the present study was to evaluate the performance of DWI in the detection of residual or recurrent disease in patients who have had a previous CWD mastoidectomy.

MATERIALS and METHODS: We identified 13 patients with a CWD mastoidectomy subsequently having at least one further DWI prior to further mastoid exploration that generated a total of 20 patient episodes. Magnetic resonance imaging was performed on a 1.5 T superconductive unit using a standard head matrix coil. Coronal 2 mm thick TSE T2-weighted images (TR: 4640 ms; TE: 103 ms; matrix: 245,384; field of view: 150×200 mm) were performed. Operative findings were reviewed for all 20 patient episodes to compare DWI findings with intraoperative findings. Based on this, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated.

RESULTS: DWI had a sensitivity of 93%, specificity of 60%, PPV of 87%, NPV of 75%, and accuracy of 80%.

CONCLUSION: Given the high sensitivity of DWI in the detection of residual or recurrent disease, the present study supports DWI as a useful tool in the detection of residual or recurrent cholesteatoma in cases following CWD surgery, where clinical acumen suggests an ongoing disease process despite no overt cholesteatoma being visible.

KEYWORDS: Diagnostic imaging, diffusion magnetic resonance imaging, diffusion-weighted magnetic resonance imaging, cholesteatoma, middle ear

INTRODUCTION

The aims of surgery in the treatment of cholesteatoma are to achieve complete clearance of cholesteatoma while causing the least disruption to the natural structures within the temporal bone. Depending on the extent of the disease, the posterior bony canal wall may be preserved, canal wall up (CWU) surgery, or removed to create a mastoid cavity, canal wall down (CWD) surgery.

With CWU surgery, residual disease can be monitored through the use of non-echo planar diffusion-weighted imaging (DWI) if second-look surgery is to be avoided. Non-echo planar DWI is currently the imaging modality of choice due to its high diagnostic performance in the detection of post-operative cholesteatoma [1-3].

In CWD cases, there is an open mastoid cavity that can be directly examined through otomicroscopy, residual or recurrent disease can often be detected and managed with microsuction in the clinic. In addition, a recent pooled analysis of level II and III studies demonstrated that CWD surgery has a lower rate of recurrent disease at 6.6%–16.5% than CWU procedures with recurrence in 12.6%–29.5% of cases [6]. Given the lower rates of recurrent disease and ability to monitor the mastoid cavity in the outpatient setting, there is presently no clear role for the use of DWI in surveillance patients following CWD surgery. Despite this, there remains a cohort of patients encoun-
tered in clinical practice who may present with symptoms suggestive of potential recurrent disease and equivocal findings on clinical examination. In this situation, imaging by non-echo planar DWI may provide additional information to guide the management of such patients.

To the best of our knowledge, there is no study that specifically explored the diagnostic performance of DWI in patients with previous CWD surgery when combined with appropriate clinical evaluation. The aim of the present study was to evaluate the performance of DWI in the detection of residual or recurrent disease in patients who have had a previous CWD mastoidectomy.

**MATERIAL and METHODS**

**Ethical Considerations**

Patients were appropriately counseled regarding their management options. We searched a prospectively updated electronic database of anonymized patient information of patients undergoing non-echo planar DWI or surgery for suspected or confirmed cholesteatoma at our institution. The database has been compiled from 2009 to 2014 and was used to identify patients who had previously undergone a CWD mastoidectomy and subsequently had non-echo planar DWI prior to a further surgical exploration of their mastoid cavity. The ethics committee of Trust approved the study.

**Patients**

We identified 13 patients with a CWD mastoidectomy subsequently having at least one further DWI prior to further mastoid exploration. A patient episode was defined as a DWI followed by a subsequent mastoid exploration in a patient who had previously had a documented CWD mastoidectomy. Of the patients, two underwent multiple mastoid explorations, each with a preceding DWI. These patients, therefore, generated multiple patient episodes suitable for analysis. A total of 20 patient episodes were generated from the 13 patients in our sample. DWI was requested pre-operatively in these patients to aid in surgical decision making.

<table>
<thead>
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<th>Indication for pre-operative DWI</th>
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<td>Incomplete ear examination (intolerable to microsuction or inaccessible cavity)</td>
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</tr>
<tr>
<td>Previous aggressive disease, DWI to assess for recurrence</td>
<td>6</td>
</tr>
<tr>
<td>New or persistent otological symptoms</td>
<td>4</td>
</tr>
<tr>
<td>Pre-operative planning</td>
<td>3</td>
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</tbody>
</table>

**Table 1. Indication for pre-operative DWI in CWD cases**

Diffusion Weighted Imaging was requested in a minority of our CWD population undergoing further mastoid exploration to aid in diagnosis and pre-operative planning. These were symptomatic patients in which a cause could not be determined clinically. This was because some patients were unable to tolerate complete clinical examination or reported new or persistent otological symptoms despite a normal clinical examination. The other patients underwent DWI to assess recurrent disease having had aggressive disease previously. A smaller cohort underwent DWI for pre-operative planning purposes. Table 1 shows the specific indication for DWI.

**Informed consent**

All patients included in the study were fully informed about the indications and risks of pre-operative imaging and subsequent surgical intervention. Verbal and written consents were obtained from the patients who participated in the study. Patients also consented for anonymized information relating to their outcomes to be used for research purposes.

**Intervention**

Magnetic resonance imaging was performed on a 1.5 T superconductive unit (MAGNETOM Avanto; Siemens Medical Solutions, Erlangen, Germany) using a standard head matrix coil. Coronal 2 mm thick TSE T2-weighted images (TR: 4640 ms; TE: 103 ms; matrix: 245,384; field of view: 150×200 mm) were also performed. In all patients, a 2 mm thick HASTE DWI sequence was acquired in the coronal plane (TR: 1600 ms; TE: 113 ms; matrix: 134×192; field of view: 220×220 mm; b factors: 0 and 1000 s/mm²). An apparent diffusion coefficient (ADC) map was calculated post-acquisition using a diffusion scan raw data. post-acquisition using a diffusion scan raw data.

All scans were reviewed by a senior radiologist (RKL) who is experienced in head and neck imaging. A diagnosis of cholesteatoma was made if the lesion demonstrated a high signal on the b0 and b1000 images and a low signal on the ADC map and T1-weighted images.

**Main Outcome Measures**

Operative findings were reviewed for all 20 patient episodes. All operations were performed by experienced otologists at our institution. The operation notes were reviewed to compare DWI findings with intraoperative findings.

**Statistical Analysis**

A descriptive analysis of sociodemographic information was performed. Performance statistics were used to calculate the accuracy, sensitivity, specificity, and negative and positive predictive values using Microsoft Excel (Microsoft, Redmond, WA, USA).

**RESULTS**

A total of 20 patient episodes were generated by 13 patients. Of the patient episodes, 90% were male, and 10% were female patients. The mean age of the patient episodes at the time of surgery was 16.69 (6.72–40.88) years. Figure 1 outlines the outcome of 20 DWI scans that had surgical correlation. The accuracy of DWI in the detection of cholesteatoma in patients with CWD was 80% in surgically confirmed cases. Table 2 shows the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of DWI.
DISCUSSION

The need of any radiological investigation is naturally dependent on its utility in influencing the overall clinical management of the patient. While the rate of residual disease following CWD mastoidectomy is lower than that following a CWU mastoidectomy, non-echo planar DWI with a sensitivity of 93% can aid in the detection of cholesteatoma in cases who are difficult to examine clinically [6]. A single false negative case was found in a patient who has a 2 mm cholesteatoma intraoperatively. This is in line with the recognized limitation of the technique of not being reliable in the detection of small cholesteatoma <2–3 mm [7, 8]. DWI can, therefore, be useful in screening post-operative patients for residual disease. Indeed, patients with CWD with known aggressive disease underwent planned DWI to detect early recurrence in our center.

Residual disease can often be detected and cleared in the clinic through micro-otoscopy of the mastoid cavity. In our patient sample, there were, however, instances where DWI was found to be valuable in guiding clinical decision making. In many patients, the entire mastoid cavity was not readily visible on micro-otoscopy due to either a high facial ridge or a polypoid disease precluding examination or patient intolerance to suction clearance (Figure 2). In addition, some patients were found on DWI to have disease extension toward the petrous apex and mastoid tip areas that are inaccessible on micro-otoscopy. In these situations, DWI aided our detection of residual disease.

Diffusion weighted imaging (DWI) can also be useful in pre-operative planning. Pre-operative imaging can characterize the extent of the disease, allowing a more tailored surgical approach. For example, a pre-operative DWI identifying a distinct pearl of keratin behind a neotympanum may allow a more limited surgical approach using endoscopic techniques, whereas a scan demonstrating extensive recurrence in yet unopened mastoid tip cells would require further mastoid surgery (Figure 3).

A mixture of wax and keratin was found intraoperatively in all of the false positive patients. This is in line with known false positive causes for DWI found in the literature [9, 10]. As wax build-up is not uncommon in an open mastoid cavity, it can cause radiological misinterpretation if the radiologist is not aware that they are dealing with an open cavity. Precise communication with radiology colleagues regarding the type of surgery performed and the middle ear cleft subsites that specifically require radiological assessment can promote optimal radiological interpretation. In addition, the use of T1-weighted images can help to improve specificity. T1-weighted images can be used to differentiate between cholesteatoma, and debris as proteinaceous fluid, inflammatory soft tissue or cholesterol granuloma can yield a high T1W signal that is not associated with cholesteatoma [7].

While the present study supports the use of DWI in the detection of residual or recurrent disease in patients with CWD, it is important to recognize the limitations of the study. As there were a total of 20 patient episodes obtained through our retrospective review of patients, a larger prospective study is required to validate these findings. In addition, our methodology involved all DWI being interpreted by a single radiologist. DWI should be reviewed by multiple radiologists to ensure inter-observer agreement in future studies.
CONCLUSION
The routine use of DWI in CWD monitoring is not warranted, but the present study supports the use of DWI in specific diagnostically difficult cases. With experienced radiological interpretation, DWI is a useful tool in the detection of residual or recurrent cholesteatoma following CWD surgery, where clinical acumen suggests an ongoing disease process despite no overt cholesteatoma being visible.

Ethics Committee Approval: Ethics committee approval was provided by the clinical governance and audit department at Northwest London Hospitals NHS Trust.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES
INTRODUCTION

The vagus nerve is the longest cranial nerve and is involved in the control of various functions of the entire body, including parasympathetic innervations to the heart, lung, and digestive organs; branchial motor functions, such as swallowing and speaking; and somatic and visceral sensations [1].

Cervical vagus nerve stimulation (VNS) has been approved for the treatment of refractory epilepsy and depression in the United States. The possible mechanisms of action include alterations in the activities of the reticular activating system, central autonomic network, limbic system, and diffuse noradrenergic projection system [2]. However, implanting a stimulator on the cervical branch of the vagus nerve is a complicated process, given the invasiveness and high cost of the procedure.

Non-invasive transcutaneous VNS (tVNS) of the auricular branch of the vagus nerve (ABVN) has been recently introduced. A functional magnetic resonance imaging (fMRI) scan has revealed that the deactivation of the limbic system—the amygdala, hippocampus, and parahippocampal gyrus—is observed after tVNS. These findings were similar to those found in the studies investigating cervical VNS [3].

OBJECTIVES: We aimed to assess the clinical significance of the intensity of transcutaneous vagus nerve stimulation (tVNS) in chronic tinnitus.

MATERIALS and METHODS: Four sessions of tVNS were performed over a 2-week period for 24 patients with unilateral, non-pulsatile chronic tinnitus. The cavum, cymba, and tragus were sequentially stimulated to the maximal sensory thresholds. One month later, after the four sessions, the level of tinnitus distress and changes in stimulus intensity were assessed.

RESULTS: The stimulus intensity did not differ according to sex or laterality. However, a moderate positive correlation between tinnitus distress and the initial stimulus intensity was observed. This correlation was not observed during the subsequent sessions. The stimulus intensity at the cavum changed significantly (p=0.018), and notable differences in tinnitus annoyance were observed between the responders and non-responders (p=0.006).

CONCLUSION: The effect of stimulus intensity on the treatment outcome seems to be limited. An increasing trend in the stimulus intensity for tinnitus annoyance at the cavum was observed in the responders. Therefore, the cavum may be an optimal stimulation site for tVNS.

KEYWORDS: Tinnitus, neuromodulation, vagus nerve, prognosis
or tensor tympani, and typewriter tinnitus), and psychiatric disorders (depression, anxiety, and obsessive-compulsive disorder) [5].

Regarding tinnitus, a promising preliminary study was recently performed to assess the combined effect of tVNS and tailor-made notched classical music [6]. The N1 m wave, which is one of the evoked auditory cortical responses during magnetoencephalography and found in some patients with sensorineural hearing loss, was attenuated, and a tendency toward decreased tinnitus distress was observed after the administration of treatment [6]. The authors stimulated the left tragus slightly above the sensory threshold using a clip-type electrode for 45–60 min each during seven sessions. Alternatively, other studies have described a stimulus intensity that was set to the highest threshold that patients could tolerate using a patch-type electrode [6]. In other studies, the intensity was simply documented as intervals from the minimal to maximal threshold without a detailed description [7, 8]. Although different criteria for stimulation intensity have been used in various studies, the clinical significance and the optimal location of the stimulation site have not been studied extensively.

In this study, we hypothesized that the maximal sensory thresholds at each tVNS site that could be tolerated without any painful sensation are associated with unique tinnitus characteristics and affect the treatment outcomes. Accordingly, we performed a prospective observational study to verify this hypothesis.

**MATERIALS and METHODS**

**Patients**

Between July 2015 and June 2016, the patients complaining of unilateral, non-pulsatile, subjective tinnitus lasting for more than 3 months and who visited the university hospital and agreed to participate were recruited for this study. In total, 24 patients (16 men, eight women; mean±standard deviation [SD] age, 44±10.93 years; age range, 24–62 years) were recruited into this prospective study. The mean duration of tinnitus was 31±49 months (range, 3–204 months).

The proposed etiologies consisted of somatosensory tinnitus after trauma in seven cases (29.2%), noise-induced hearing loss in six cases (25.0%), sudden deafness in four cases (16.7%), Meniere’s diseases in three cases (12.5%), presbycusis in two cases (8.3%), acoustic trauma in one case (4.2%), and the undiagnosed in one case (4.2%).

The exclusion criteria were as follows: bilateral and/or pulsatile tinnitus, tinnitus under medical treatment, previous diagnosis of neuropsychiatric or dermatological diseases, and the presence of an implanted metal device in the cranial region. The Institutional Review Board of Eulji University approved this study. Written informed consent was obtained from all the patients.

**Study Design**

The overall study design is depicted in Figure 1. During the initial visit, the patients were screened and the severity of tinnitus was assessed using questionnaires, including the Tinnitus Handicap Inventory (THI), the Beck Depression Inventory (BDI), and a visual analog scale (VAS) from 0 to 10 (0: no symptoms, 10: maximal symptoms) regarding tinnitus loudness (LD), awareness (AW), annoyance (AN), and its effect on life (EL).

During the second visit, an audiological assessment comprising pure tone audiometry, speech audiometry, tinnitography, otoacoustic emissions, and test of auditory brainstem response was performed. The first tVNS was subsequently performed. All patients participated in the remaining three sessions during the consecutive visits over a 2-week period.

Following a 4-week washout period, the patients’ subjective distress regarding tinnitus was re-assessed using questionnaires. Patients whose final VAS score decreased by 50% or more were categorized as responders, whereas others were considered as non-responders.

**The tVNS Protocol**

tVNS was performed using a transcutaneous electrical nerve stimulation (TENS) device (ES-420, Ito Co., Ltd., Tokyo, Japan). The stimulation conditions were as follows: pulse width, 200 μs; frequency, 30 Hz; stimulation sites (in sequence), the cavum, cymba, and the outer surface of the tragus; and the duration of stimulation, 4 min for each site (Figure 2) [7]. A ball-type electrode was placed on the stimulation site, and the intensity was increased by 1 mA every 5 s until the maximum intensity that the patients could tolerate without feeling pain.

**Statistical Analysis**

The two-tailed Fisher’s exact test was performed to assess the differences between the categorical variables. The changes in VAS, THI, and BDI scores after treatment were compared using the Wilcoxon signed-rank test. The correlations between the stimulus intensity and VAS were assessed using the Spearman correlation analysis. Repeated measures analysis of variance (ANOVA) was used to assess the differences in the stimulus intensity at each stimulation site over time. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software version 24.0 (IBM Corp, Armonk, NY, USA) for Macintosh (Apple Inc., Cupertino, CA, USA); p<0.05 was considered statistically significant.

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**Figure 1. Schematic overview of the study design.**

tVNS: transcutaneous vagus nerve stimulation.
RESULTS

Patient Characteristics
Of the 24 patients, 16 complained of left-sided tinnitus and eight complained of right-sided tinnitus. The mean hearing threshold was 26 ± 22 dB in the affected side and 18 ± 21 dB in the unaffected side; this difference was not statistically significant (p > 0.05). The mean THI and BDI scores were 45 ± 19 and 12 ± 11, respectively.

Differences in Stimulus Intensity According to Sex, Laterality, and Stimulation Site
The stimulation intensity did not show any significant difference with respect to sex and laterality (p > 0.05). With regard to the stimulation site, the mean maximal sensory threshold at the tragus was 5.79 ± 2.39 mA (range, 1–14 mA); this tended to be higher than that at the cymba (4.98 ± 2.09 mA; range, 1–14 mA) or cavum (5.10 ± 1.86 mA, range 1–12 mA; p = 0.018).

Changes in VAS, THI, and BDI Scores After Treatment
All the VAS scores indicating tinnitus distress improved after the treatment (p < 0.05) (Figure 1). In addition, the THI and BDI scores were significantly reduced to 27 ± 15 (p < 0.001) and 9 ± 11 (p = 0.004), respectively. Based on the VAS scores, 33.3% (n = 8), 62.5% (n = 15), 45.8% (n = 11), and 41.7% (n = 10) of the patients were classified as responders based on LD, AW, AN, and EL, respectively.

Relationship Between Stimulus Intensity and Tinnitus Severity
The repeated measures ANOVA revealed that the stimulus intensity at the cavum differed significantly across sessions (p = 0.018). Conversely, no significant change was observed at the cymba or tragus (p > 0.05). Moreover, the sum of the stimuli at all stimulation sites did not change significantly (p > 0.05).

At the cavum, a moderate positive correlation was observed between stimulus intensity and all VAS scores, except EL, in the first session (LD: r = 0.57 and p = 0.004; AW: r = 0.48 and p = 0.017; and AN: r = 0.50 and p = 0.011). However, only AW showed a moderate positive correlation in the second session (r = 0.51 and p = 0.011). No significant correlation was found between the VAS scores and stimulus intensity even in the third and fourth sessions. In addition, these trends were similar at both the cymba and tragus (data not shown).

The VAS scores of the responders were subsequently compared. The stimulus intensity at the cavum showed a significant difference between the responders and non-responders in terms of AN (Figure 3; p = 0.006). However, no differences were observed at the other stimulation sites or in the VAS scores.

Lastly, the changes in THI and BDI scores and the percentage changes in the VAS scores between the initial and final observations were
Subsequently, the relationship between these scores, stimulation intensity at each site, and sum of stimuli at all sites were assessed using the Spearman’s correlation analysis. No significant correlation was observed between the stimulation intensity at each site and in the changes in the scores. Similarly, the sum of stimuli at all sites was not correlated to the scores, irrespective of the response to treatment (p>0.05).

### Adverse Effects
None of the patients complained of chest pain, dizziness, headache, otalgia, or dermatological effects at the stimulation sites.

### DISCUSSION
Although few previous studies have evaluated the feasibility of tVNS for treatment of chronic tinnitus, the present study is the first to evaluate the effect of the stimulus intensity of tVNS on the treatment of chronic tinnitus. In this study, we investigated the effect of stimulus intensity on the treatment outcome, which has been frequently overlooked in many previous studies, and identified the optimal stimulation site.

Based on our findings, the effect of stimulus intensity on the treatment outcome seems to be limited, although a positive correlation exists between the initial VAS scores and the stimulus intensity. We conclude that the stimulus intensity may be subsidiary to whether tVNS should be performed.

From an anatomical perspective, there are heterogeneous distributions of nerves in the external ear; the cymba is innervated by ABVN, both ABVN and the greater auricular nerve (GAN) are found in the cavum, and at the tragus, three nerves, ABVN, GAN, and auriculotemporal nerve are observed at the tragus. A recent imaging study reported that stimulating the left cymba induced a widespread activation from the ipsilateral nucleus tractus solitarii (NTS) to the brainstem and to the forebrain along the central vagal afferent pathway. Consistently, other studies have proposed a hypothesis for the

### Table 1. Demographic information and the treatment outcome for tinnitus patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Onset (months)</th>
<th>Side</th>
<th>First session</th>
<th>Second session</th>
<th>Third session</th>
<th>Fourth session</th>
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<th>ΔBDI</th>
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CB: cymba concha; CC: cavum concha; T: tragus; SUM CB + CC + T; Δ: the change between initial and final measurement; THI: tinnitus handicap inventory; BDI: Beck depression inventory; LD: loudness; AW: awareness; AN: annoyance; EL: effect on life.
mechanism underlying the therapeutic effect of tVNS; it may originate from the noradrenergic and serotonergic interactions between the NTS, locus coeruleus (LC), and raphe nuclei [16].

Alternatively, other studies have compared the four stimulation sites in healthy subjects—the inner tragus, inferoposterior wall of the external auditory meatus, cymba, and ear lobe—to ascertain the optimal tVNS site [12]. The authors found that the stimulation of the cymba produced a stronger activation in the NTS and the LC than at the ear lobe (sham), although they also reported a contradictory result that the stimulation of the ear lobe deactivated the Heschl’s gyrus and the superior temporal gyrus, similar to a real stimulation. Collectively, these results suggest that the optimal site for tVNS is, in theory, the cymba.

Conversely, in the present study, the responders could not be differentiated from non-responders using the cymba concha stimulation. Instead, the stimulus intensity showed a significant change at the cavum and the responders exhibited a changing pattern of stimulus intensity across sessions (Figure 3), which was significantly different from our expectations.

With regard to the anatomical perspective, the cavum is innervated by both the ABVN and GAN, whereas the ear lobe, which has been frequently used as a sham stimulation site in many tVNS studies, is innervated by GAN alone. GAN is the superficial branch of the cervical plexus from the C2 and C3 spinal nerves and is divided into anterior and posterior branches. The anterior branch runs into the parotid gland, whereas the posterior branch communicates with ABVN and the posterior auricular branch of the facial nerve [13]. Moreover, a previous study reported that TENS of the C2 dermatome may enhance the inhibitory role of the dorsal cochlear nucleus (DCN) in the central auditory pathway in cases where tinnitus could be modulated by somatosensory events [14]. Collectively, these data led us to conclude that the anatomical interconnection between GAN and ABVN as well as the auditory-somatosensory integration in DCN explain both our findings and the similarity between the fMRI findings for sham and real stimulations [12].

We found that the initial stimulus intensity correlated the most with the VAS scores; however, the correlation was not observed after multiple sessions. The initial correlation may provide evidence supporting a therapeutic effect of tVNS or it may reflect the patients’ expectations for improvement in proportion to subjective tinnitus distress. In addition, the lack of correlation between stimulus intensity and final VAS scores may arise from early onset tolerance due to repeated administration of tVNS [15].

In general, sensory intensity is defined as the point at which patients feel a strong but tolerable sensation without a motor contraction [16]. Thus, the threshold used in this study corresponded to “maximal” sensory stimulus intensity. The frequency is also an important factor; >100 Hz is regarded as high-frequency stimulation and <10 Hz is regarded as low-frequency stimulation [16]. In the current tVNS studies, including the present study, a low-frequency (<30 Hz) was used [8-10, 12, 14].

Apart from the modulating options of frequency and/or intensity, commercially available tVNS devices are also equipped with “on” and “off” functions. The options to adjust the stimulation patterns or waveforms are also available in most TENS devices. A recent study reported that alternating low and high frequencies were helpful in slowing the development of tolerance in arthritic rats [17]. Thus, the modulating stimulation patterns may be an important approach for preventing tolerance.

This study has several limitations. Firstly, four separate 12-min sessions appeared to be inadequate for an outcome assessment. However, because we also wanted to ascertain the optimal stimulation site for the treatment of tinnitus, we selected three tVNS application sites based on the related literature review. Further studies with longer sessions devoting more time to the cavum are needed. Secondly, the study design did not include a control group, and this may have increased the possibility of bias. Thirdly, we did not document the changes in the VAS scores directly before or after every session. If we had compared the VAS scores with stimulus intensity at every session, more details regarding the relationship between the intensity and VAS could have been confirmed. Finally, fMRI or positron emission tomography were not performed. Although these techniques do not always provide direct information regarding tinnitus patients, neural activities may be measured indirectly by detecting changes in perfusion or local metabolism, and this may have provided additional information for our study. Lastly, the effect of tVNS may be influenced by age. A previous study has suggested that sedentary or old populations reduced parasympathetic activity compared with healthy participants [18]. However, only two of the patients included in this study were of 60 years of age or older. Thus, our results may not be greatly affected by aging.

CONCLUSION
The stimulus intensity for tVNS does not differ according to sex or laterality. Although a moderate, positive correlation between intensity and tinnitus distress was noted initially, it did not persist after subsequent sessions. Therefore, the effect of stimulus intensity on the treatment outcome seems to be limited. Among patients with AN, the responders to tVNS showed a small increasing trend in stimulus intensity at the cavum. Conversely, no significant differences according to treatment response or other VAS scores were found at the cymba or tragus. Therefore, these results suggest that the optimal stimulation site for tVNS is the cavum and not the cymba.

Ethics Committee Approval: The Institutional Review Board of Eulji University approved this study (IRB 2015-04-012).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.


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Conflict of Interest: The authors have no conflict of interest to declare.

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INTRODUCTION

Despite years of research on the topic, little is known about tinnitus, which is defined as the perception of sound without any external source. Nearly 20% of the world’s population suffers from tinnitus, and according to a recent study, at least one-fourth of this group has reported interference with daily activity and quality of life [1]. With estimated treatment costs exceeding $345.5 million per year in the United States alone, tinnitus has also resulted in massive financial burden [2]. Furthermore, evidence has indicated that patients with tinnitus can suffer mental health problems.

The pathophysiology of tinnitus is complicated, and its distinct etiology can only be found in a limited number of patients. Numerous types of treatment modalities, such as oral medication, retraining therapy, or transtympanic laser therapy, have been attempted [3]. However, because of the unclear etiology of tinnitus in most cases, clinicians often fail to constitute a proper algorithm that can effectively treat it.

Because the clarification of tinnitus’ pathogenesis plays a key role in the effective treatment of the disease, studies should focus on tinnitus’ possible etiological factors in order to achieve satisfactory treatment results. This study aimed to evaluate certain inner ear structures in patients who suffered from tinnitus, hypothesizing that regardless of the presence of hearing loss, tinnitus may occur when the bony structures that surround the cochlear nerve become narrower.

OBJECTIVE: The objective of this research was to investigate the possible relationship between tinnitus and certain bony inner ear structures using computed tomography (CT).

MATERIALS and METHODS: This was a prospective, controlled, double-blind study. The subjects of the study were divided into the following three groups: group 1 (G1), patients with unilateral sensorineural hearing loss (SNHL) and unilateral non-pulsatile tinnitus in the same ear; group 2 (G2), patients with normal hearing and unilateral non-pulsatile tinnitus; and group 3 (G3), healthy volunteers with neither tinnitus nor hearing loss. The basal turn length, internal acoustic canal (IAC) width and length, bony cochlear nerve canal (BCNC) width, and IAC diameter at the porus acusticus internus (PAI) were measured.

RESULTS: The mean BCNC width was significantly narrower in G1 and G2 than in the control group (G3) (p<0.001). For patients in G2, BCNC width was significantly narrower in ears with tinnitus (p<0.001) than in ears without tinnitus. The mean IAC diameter at PAI was also narrower in the G1 patients (p=0.007) compared with the other groups.

CONCLUSION: The results of this study suggest that CT evaluation of the inner ear structures is important in patients with tinnitus. According to the results, a narrow BCNC may cause phantom sensations and be related to cochlear nerve dysfunction. Therefore, it is recommended that clinicians evaluate BCNC carefully while assessing such patients.

KEYWORDS: Tinnitus, temporal bone, computed tomography, bony cochlear nerve canal
MATERIALS and METHODS

Subjects and Clinical Setup
This prospective, controlled, double-blind study was conducted in a tertiary referral hospital, and all clinical examinations and radiological evaluations were performed in the departments of radiology and otolaryngology. Each patient signed an informed consent form before the study began, and the Declaration of Helsinki’s ethical principles on human experimentation were followed. The study was approved by the ethics committee (EC) of Keçiören Research and Training Hospital (EC No: 868).

Patients with and without hearing loss were included in the study if they had been admitted to the otolaryngology outpatient clinic with complaints of unilateral non-pulsatile tinnitus. Patients with a Tinnitus Handicap Inventory (Turkish Validated) score <76 were included in this study (4). Patients were excluded from the study if they used chronic medications, such as acetylsalicylic acid; had histories of malignancies, otologic surgery, and prior otitis media/externa; were using hearing aids; or had psychiatric disorders, abnormal otoscopic findings, accompanying vestibular complaints, chronic systemic and/or vascular diseases, and the presence of conductive types of hearing loss or abnormal tympanometry findings. All study subjects were given a detailed clinical examination by the same otolaryngologist. Audiological status was assessed with tympanometry and pure tone audiometry (PTA). Inner ear structures were evaluated with temporal bone computed tomography (CT).

The subjects were divided into the following three groups: group 1 (G1), patients with unilateral sensorineural hearing loss (SNHL) and unilateral non-pulsatile tinnitus in the same ear; group 2 (G2), patients with normal hearing and unilateral non-pulsatile tinnitus; and group 3 (G3), age- and gender-matched patients with neither tinnitus nor hearing loss who had been scanned for reasons other than otologic issues. G3 served as the control group for G1 and G2. While the bony inner ear structures were only measured in the affected ears of the subjects in G1, the bony inner ear structures were measured in the right ears of every subject in G3. Both ears of the patients in G2 were evaluated to compare the structures of both sides within the group. All patients in G1 and G2 also underwent magnetic resonance imaging (MRI) to exclude the presence of intracranial lesions, such as acoustic neuromas. Patients with any pathologies in the cerebellopontine angle or internal auditory canal were also excluded from the study.

Audiological Assessment
For the audiological evaluation, PTA was performed using the Orbiter 922® Clinical Audiometer (Madsen Electronics, Copenhagen, Denmark) for frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz. A 226-Hz tympanometry was performed using an AT235® impedance audiometer (Interacoustics, Copenhagen, Denmark) device. All patients were assessed by the same audiometrist. Subjects with a mean hearing threshold of <26 dB hearing level (dBHL) were considered to have normal hearing (5).

Radiological Evaluation
All patients were evaluated by the same radiologist who had been blinded to the clinical data. In total, 74 temporal bone CT examinations were performed for G1 and G2. For G3, 41 temporal bone

CT examinations were performed on 41 patients who had been scanned for head trauma. All CT scans were performed with a 16-row multi-detector CT scanner (Somatom Emotion; Siemens Healthcare, Forchheim, Germany). The CT parameters of the temporal bone scan included the following: 0.75 s of gantry rotation time, 110 kVp, 120 mA, 0.6 mm section thickness, 16×1.2 detector configuration, and 512×512 matrix size.

The bony cochlear nerve canal (BCNC) width was measured at the fundus level of internal acoustic canal (IAC) to the mid-modiolar cochlea in the axial images (Figure 1). IAC length was measured from the imaginary mid-point of the porus to the transverse crest, and IAC width was measured at the mid-canal level from the coronal reformatted CT images of the temporal bones of the subjects (Figure 2). The IAC diameter at the porus acusticus internus (PAI) and the basal turn length of the cochleae were also measured using the axial images.

Statistical Analysis
Fisher's exact test and Pearson’s chi-square test were used to compare the groups. For more than two groups, analysis of variance (ANOVA) was used to evaluate the significance of the differences in the averages between the groups. Statistical Package for Social Sci-
ences version 15.0 for Windows (SPSS Inc.; Chicago, IL, USA) was used for all statistical analyses, and a p value of <0.05 was considered to be statistically significant.

RESULTS

The study included 115 patients. There were 36, 38, and 41 patients in G1, G2, and G3, respectively. No inner ear anomalies were detected in any of the groups. Because of the study criteria, one patient was excluded when an osteoma was found at the porus of IAC. Measurements of the IAC width and length, BCNC width, IAC diameter at PAI, and basal turn length are summarized in Table 1. According to ANOVA, the mean BCNC width was significantly narrower in patients in G1 and G2 than in those in G3 (p<0.001) (Figure 3). The IAC diameter at PAI was also smaller in G1 than in G2 and G3 (p=0.007). The measurements of the IAC width and length and the basal turn length were similar between the groups.

Table 2 shows a comparison between the affected and unaffected ears of G2, wherein the unaffected ears serve as the control group for ears with tinnitus. According to Table 2, the BCNC width was significantly narrower in ears with tinnitus than in unaffected ears (p<0.001). The measurements of IAC diameter and length and the basal turn length were similar between the affected and unaffected ears.

DISCUSSION

Because tinnitus remains to be one of the most common neurological disorders worldwide, numerous studies have been published to better understand its mechanisms and develop new strategies for its treatment. A small proportion of patients have exhibited few pathologies, such as glomus tumors and acoustic neuroma, which directly relate to tinnitus. Ruling out the limited number of patients who have had obvious pathologies, a vast majority of people with tinnitus remain untreated and undiagnosed.

Because tinnitus’ etiology is key to its accurate treatment, clinicians have focused on research that has investigated the disease’s possible pathogenetic mechanisms. As a result, otological diseases have been studied alongside hormonal changes, physiological diseases, neurological disorders, cardiovascular diseases, intracranial pathologies, and temporomandibular joint disorders [6]. Regardless, a majority of patients have exhibited no obvious etiological explanations for tinnitus in spite of diagnostic testing. These patients have primarily been treated with oral betahistine, anti-depressants, or tinnitus retraining therapy.

REFERENCES

Do not hallucinate.
MRI. While MRI is a valuable diagnostic technique for assessing soft tissue, it has limited accuracy in its ability to evaluate bony structures. The most appropriate radiological imaging option for investigating bony inner ear structures is temporal bone CT. Evidence has suggested an association between SNHL and narrowed inner ear structures, such as BCNC [7,8]. BCNC is described as a bony transition canal that is located between IAC and cochlear modiolus (Figure 4) [9]. In a radiographic study of BCNC by Stjernholm and Murren, a value of 1.4 mm was suggested to indicate hypoplasia of BCNC [10]. A similar value was determined in the present study.

By broadening this viewpoint so that it did not focus solely on BCNC, which is the narrowest point of the nerve course, we hypothesized that the compression of certain inner ear structures, such as IAC or PAI, may produce symptoms of tinnitus regardless of the presence of hearing loss. After excluding subjects who exhibited factors that may cause tinnitus, a group of patients who showed signs of unilateral subjective non-pulsatile tinnitus was formed. Subgroups were formed according to hearing status. The significant narrowness of BCNC in G1 and G2 might result in the dysfunction of the eighth cranial nerve. Because we asserted that a hypoplastic BCNC may be related to the development of SNHL, this relationship is referred to as the dysfunction of the eighth cranial nerve.

The coronal CT images revealed no differences in the IAC lengths and widths between the groups (Table 1). These values were also similar between the ears of patients in G2 (Table 2). The basal turn length was also similar between the groups. We measured this location because it is one of the most reliable markers that can indicate an anatomically normal cochlea. However, because the presence of a competent cochlea is essential to an intact acoustic pathway, we measured the basal turn length in all patients to evaluate their cochleae radiologically. Another remarkable structure in the cochlear nerve pathway is PAI. The IAC diameter at PAI was measured in all patients and was found to be wider in G2 and G3 than in G1. The narrowness of this structure in G1 was statistically significant, leading to the conclusion that the compression of the eighth cranial nerve at any point could result in tinnitus or related symptoms.

In recent years, there has been an increase in the number of studies that evaluate the association between cochlear nerve function and bony inner ear structures. For instance, Kumral et al. [11] found no anatomical differences in the etiology of tinnitus when they studied the inlet, mid-canal, and outlet measurements of IAC in patients who had and did not have tinnitus. In a study of 51 patients with unilateral SNHL, Yi et al. [9] found that the BCNC diameter was significantly smaller in ears with SNHL than in normal ears. They found narrow BCNCs in more than half of the ears with SNHL, which indicated a significant association between BCNC stenosis and cochlear hypoplasia. In the presence of normal cochleae, BCNC stenosis has also been reported to be a potential cause of congenital SNHL [12-14]. The studies that arrived at this conclusion typically referred to BCNC as a potential anatomical site for the evaluation of bony inner ear structures with temporal bone CT.

This study was consistent with existing literature because no correlation was found between tinnitus and IAC length and width. The basal turn length was also similar between the study and control groups. According to this study’s data, the mean BCNC diameter was significantly smaller in patients with tinnitus than in patients without tinnitus. This significance was present regardless of the patients’ hearing status. Recent studies have indicated an association between SNHL and BCNC stenosis. By discussing the relationship between tinnitus and BCNC tinnitus, the results of this study will bring a new perspective to research on tinnitus.

The compression of neurovascular structures may cause various symptoms, such as neuralgia, hypoesthesia, and even phantom sensations. Although not all cases of neurovascular compressions are symptomatic, a number of clinical syndromes have been detected within the cranial nerves. These syndromes include hemifacial spasm, trigeminal neuralgia, vestibular paroxysmia, and glossopharyngeal neuralgia. The compression of neural structures, especially as they pass through the foramina and bony canals, may cause the abovementioned symptoms. The positive relationship between patients with tinnitus and narrow BCNCs may also be an example of this phenomenon [15].

This study was restricted by its limited number of participants. Further studies that contain larger groups and that match this study’s criteria will provide more information about the relationship between tinnitus and bony inner ear structures. Audiatory brainstem responses or otoacoustic emissions could have been performed to evaluate the integrity of the cochlear nerve and hair cell damage; however, because the vast majority of the study’s subjects were adult patients who had normal hearing during childhood, PTA was found to be sufficient for the audiological assessment.

CONCLUSION
The findings of this study suggest that CT evaluation of the inner ear structures is important in patients with tinnitus, particularly for those who have experienced hearing loss. According to the results, narrow BCNCs may be related to dysfunction of the cochlear nerve, which in turn, may result in tinnitus. It is therefore recommended that clinicians evaluate BCNC carefully while assessing patients with tinnitus.

Ethics Committee Approval: Ethics committee approval was received for this study from Keçiören Clinic Research Ethics Committee (Approval Date: 12.04.2016/Approval No: 868).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.
REFERENCES
INTRODUCTION
Cancers of the external auditory canal (EAC) are rare, with an estimated incidence of less than 0.2% of all head and neck cancers [1]. Representative symptoms are otorrhea, otalgia, hearing impairment, and external visible mass. It is frequently misdiagnosed owing to its similar clinical features to benign conditions, such as granulation tissue formation in the EAC, EAC cholesteatoma, and chronic otitis externa. The most common histological type of EAC malignancy is squamous cell carcinoma, followed by adenoid cystic carcinoma, basal cell carcinoma, malignant melanoma, Merkel cell carcinoma, angiosarcoma, adnexal carcinoma, ceruminous adenocarcinoma, and lymphoma [1-3].

Since 1970s, the survival rates of carcinoma of the EAC (CEAC) have improved owing to the development of microsurgery and advanced diagnostic imaging systems, such as computed tomography (CT) and magnetic resonance imaging (MRI). Various previ-
ous studies have reported the survival rates of CEAC to be from 15% (advanced stage) to 100% (early stage), depending on the extent of the disease [1, 2, 4-6]. The prognosis seems to be influenced by the histological type of cancer, local or distant recurrence, and lymph node invasion [4, 7, 8].

To date, there is no consensus on the guideline for the treatment of EAC malignant tumors, and the optimal management of patients is still a debatable topic. In the present study, the authors collected data of patients with CEAC from four tertiary hospitals and analyzed the surgical outcomes related to the stage, adjuvant therapy, and simultaneous surgeries to suggest treatment options for patients with CEAC.

MATERIALS AND METHODS

Study Population
Data of 33 patients who were diagnosed with and surgically treated for CEAC in 2009–2016 were collected from four tertiary multicenter hospitals for retrospective analysis. Data closing date of the study was December 31, 2017. Medical records and radiological findings from CT and/or MRI were reviewed to determine cancer stage according to the Pittsburgh classification (Figure 1, Table 1). Of the 33 patients, 2 were excluded because of premature follow-up loss, i.e., follow-up of less than 1 month; therefore, 31 patients were enrolled in this study. All patients underwent surgical treatments, such as lo-

Figure 1. a-d. Representative case of cancer of the external auditory canal. (a) Axial non-enhanced CT scan. Soft tissue obliterates in right external auditory canal. (b) Axial T1-weighted MRI image. The main tumor shows a heterogeneous intermediate signal intensity. (c) Specimen (size, 1.8×1.5 cm). (d) Histological section (H&E). Histological section of the specimen shows a well-differentiated squamous cell carcinoma characterized by hyperchromatic nuclei and keratin pearl formation.
Table 1. Pittsburgh staging system for carcinoma of the external auditory canal

T status

T1 Tumor limited to the external auditory canal without bony erosion or evidence of soft tissue involvement
T2 Tumor with limited external auditory canal bony erosion (not full thickness) or limited (<0.5 cm) soft tissue involvement
T3 Tumor eroding the osseous external auditory canal (full thickness) with limited (<0.5 cm) soft tissue involvement or tumor involving middle ear and/or mastoid
T4 Tumor eroding the cochlea, petrous apex, medial wall of middle ear, carotid canal, jugular foramen or dura, with extensive (≥0.5 cm) soft tissue involvement, such as involvement of temporomandibular joint or styloid process, or evidence of facial paresis

N status

Lymph node involvement is a poor prognostic sign; any node involvement should automatically be considered as advanced stage (i.e., T1N1=stage III and T2, 3, 4 N1=stage IV)

M status

Distant metastases indicate a very poor prognosis and should be considered as stage IV

In the absence of metastatic lymph nodes or distant metastases, T status of the tumor defines the clinical stage

Results

The mean age of the enrolled patients was 67 years (range, 44–84 years), with gender distribution of 13 males (41.9%) and 18 females (58.1%). The right-sided tumors were 16 cases (51.6%) and the left sided tumors were 15 cases (48.4%). The most common representative symptom was otorrhea (n=16), defined as any discharge from EAC, followed by ear fullness (n=11), hearing impairment (n=5), and visible mass (n=3) (Table 2). According to the Pittsburgh TNM stage, at initial diagnosis, 22 patients were in the early stages (stage I: 15; stage II: 7) and 9 were in the advanced stages (stage III: 1; stage IV: 8). The most common histological type was squamous cell carcinoma (n=20). The detailed demographic data of study subjects are shown in Table 3.

All patients received surgical treatments: local resection (n=15; 48.4%), temporal bone resection (n=13; 41.9%), and subtotal petrosectomy (n=3; 9.7%). Pathological evaluations of the resected tissues showed negative surgical margins in all patients, and 1 patient (Case No. 30) with the closest tumor margin of 2 mm underwent a secondary resection. Simultaneous parotidectomy was performed in all patients except for patients with local resection (n=15) and subtotal petrosectomy (n=1). Simultaneous neck dissection was performed in 11 patients. One patient (Case No. 22), who was clinically N0 before the surgery, showed a level II metastatic lymph node during the postoperative pathological evaluation and was transferred to the oncology department for adjuvant RT. Two patients showing pathologically direct invasion to the parotid glands were also clinically diagnosed before the surgery. No patient showed an occult metastasis in the intraparotid lymph node. An analysis of simultaneous surgical resection of simultaneous neck dissection showed a trend toward a better survival rate among two groups (early stages vs advanced stages) were compared using Kaplan–Meier method. A two-tailed p<0.05 was considered statistically significant.

Ethics Considerations

The study protocol and procedures were reviewed and approved by the Institutional Review Board of our institution (IRB No. XC16RIMI00043), which did not request informed consent for this retrospective study.

Table 2. Initial symptoms of patients with cancer of the external auditory canal

<table>
<thead>
<tr>
<th>Initial symptoms</th>
<th>Number of patients (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otorrhea</td>
<td>15 (48.4%)</td>
</tr>
<tr>
<td>Ear fullness</td>
<td>5 (16.1%)</td>
</tr>
<tr>
<td>Visible mass</td>
<td>5 (16.1%)</td>
</tr>
<tr>
<td>Hearing impairment</td>
<td>2 (6.5%)</td>
</tr>
<tr>
<td>Facial palsy</td>
<td>2 (6.5%)</td>
</tr>
</tbody>
</table>
survival rate (p=.053, Log Rank). However, simultaneous parotidectomy did not seem to influence the survival (p=.127, Log Rank).

Postoperative complications including facial palsy (n=4), surgical wound dehiscence (n=3), wound infection (n=2), and dizziness (n=1) appeared in 10 of 31 patients (32.3%) (Table 4). All patients with postoperative facial palsy developed their symptom of facial weakness after the surgical treatment that included parotidectomy. Of these 4 patients, 3 patients showed House-Brackmann (H-B) grade II facial

Table 3. Baseline characteristics of 31 patients operated for cancer of the external auditory canal

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Side</th>
<th>Stage</th>
<th>Pittsburgh TNM</th>
<th>Histopathology</th>
<th>Surgical Type</th>
<th>Adjuvant therapy</th>
<th>Recurrence</th>
<th>F/U (mo)</th>
<th>Patient status</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>M</td>
<td>L</td>
<td>II</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>LTBR, P, SND</td>
<td>-</td>
<td>-</td>
<td>41</td>
<td>A</td>
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<tr>
<td>2</td>
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<td>F</td>
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<td>LTBR, P</td>
<td>RT</td>
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<tr>
<td>3</td>
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<td>LCR</td>
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<tr>
<td>4</td>
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<td>LCR</td>
<td>-</td>
<td>-</td>
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<td>T1N0M0</td>
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<td>LCR</td>
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<td>-</td>
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<td>LCR</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>IV</td>
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<td>LTBR, P, SND</td>
<td>RT</td>
<td>Local/reOP</td>
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<td>LCR</td>
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<td>SCC</td>
<td>LTRB, P</td>
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<td>-</td>
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<td>II</td>
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<td>SP</td>
<td>RT</td>
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<td>A</td>
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<td>AC</td>
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<td>CRT</td>
<td>-</td>
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<tr>
<td>20</td>
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<td>R</td>
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<td>T3N1M0</td>
<td>SCC</td>
<td>SP, P, SND</td>
<td>RT</td>
<td>Distant</td>
<td>8</td>
<td>D</td>
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<td>II</td>
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<td>STBR, P, SND</td>
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<td>-</td>
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<td>BCC</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<td>28</td>
<td>67</td>
<td>M</td>
<td>R</td>
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<td>T2N1M0</td>
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<td>LTBR, P, SND</td>
<td>RT</td>
<td>-</td>
<td>16</td>
<td>D</td>
</tr>
<tr>
<td>29</td>
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<td>R</td>
<td>II</td>
<td>T2N0M0</td>
<td>SCC</td>
<td>LCR</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>A</td>
</tr>
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<td>30</td>
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<td>M</td>
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<td>LCR</td>
<td>RT</td>
<td>Distant</td>
<td>47</td>
<td>A</td>
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<tr>
<td>31</td>
<td>70</td>
<td>F</td>
<td>L</td>
<td>I</td>
<td>T1N0M0</td>
<td>BCC</td>
<td>LCR</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>A</td>
</tr>
</tbody>
</table>

SCC: squamous cell carcinoma; BCC: basal cell carcinoma; ACC: adenoid cystic carcinoma; VC: verrucous carcinoma; AC: adenocarcinoma; LCR: local canal resection; LTBR: lateral temporal bone resection; STBR: subtotal temporal bone resection; SP: subtotal petrosectomy; P: parotidectomy; SND: selective neck dissection; MRND: modified radical neck dissection; RT: radiation therapy; CRT: chemoradiation therapy; A: alive; D: died.

Table 4. Postoperative complication rate of patients with cancer of the external auditory canal

<table>
<thead>
<tr>
<th>Complication type</th>
<th>Number of patients (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial palsy</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>Surgical wound dehiscence</td>
<td>3 (9.7%)</td>
</tr>
<tr>
<td>Wound infection</td>
<td>2 (6.5%)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1 (3.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>10 (32.3%)</td>
</tr>
</tbody>
</table>

The survival rate (p=.053, Log Rank). However, simultaneous parotidectomy did not seem to influence the survival (p=.127, Log Rank).

Postoperative complications including facial palsy (n=4), surgical wound dehiscence (n=3), wound infection (n=2), and dizziness (n=1) appeared in 10 of 31 patients (32.3%) (Table 4). All patients with postoperative facial palsy developed their symptom of facial weakness after the surgical treatment that included parotidectomy. Of these 4 patients, 3 patients showed House-Brackmann (H-B) grade II facial
One patient with H-B grade V facial palsy underwent facial nerve decompression after 1 month of initial surgery, but the outcome was not successful. The median follow-up period of enrolled patients was 16 months (range, 4–95 months). After the initial surgical treatment, 5 patients (16.1%) showed recurrence; of them, 2 patients with local recurrence were re-operated and 3 patients with distant recurrence were transferred to the oncology department for further management. Finally, 2 recurred patients died during the study period. During the follow-up period, 1 stage-II patient and 3 stage IV patients died even after undergoing adjuvant therapy after the surgery. Although early-stage patients showed 100% 5-year cumulative survival rate, the advanced-stage patients showed 5-year survival rate of 53.6%, which is a statistically significant difference (p=.006) (Figure 2). The overall survival rate of all enrolled patients was 90.3%. The 5-year disease-free survival rate of each stage was 100.0% for both stages I and III, 30.0% for stage II, and 72.9% for stage IV (Figure 3). Low disease-free survival rate of stage-II patients attributes to 1 local recurrence and 2 distant recurrences of a total of 7 patients.

DISCUSSION

The prevalence of CEAC is estimated to occur in 1-6 people per 1 million population, with a mean age of 61.5 years. Sex ratio is reported to be equal [1-3, 9, 10]. Due to its non-specific clinical symptoms, such as otorrhea, otalgia, and hearing loss, it is often difficult to be detected at early stage. According to a previous report, the misdiagnosed rate of CEAC is up to 69% [1]. Therefore, thorough physical examination, including tympanic endoscopy and full diagnostic imaging, is essential for the early diagnosis of CEAC. CT scans are one of the most reliable methods for the identification of CEAC because it is convenient to find bony erosions of the EAC and determine the extent of the disease. According to Leonetti et al. [11], comprehensive assessment of CT scans strongly correlate with the actual operative findings. However, CT scans cannot accurately detect soft tissue invasion or mucosal thickening without bony erosion and therefore must be supplemented with MRI. Preoperative MRI is particularly important in an advanced CEAC stage because neurovascular invasion and spread into cranial fossae are better detected in a fat-signal suppressed enhanced T1-weighted MRI images than in CT [12]. In addition to CT and MRI scans, positron emission tomography scans may also be considered for detecting invasions to nearby anatomic structures, such as the parotid gland, as well as metastasis to distant organs. As for the staging of CEAC, the University of Pittsburgh Staging System modified by Moody et al. [13] has been demonstrated to be reliable and reproducible in the international literatures since the beginning of the 1990s. Squamous cell carcinoma is the most commonly observed tumor of the EAC and accounts for 80% of the tumors of the temporal bone [10-17]. The survival rate for this type of cancer is poorer than that for adenoid cystic carcinoma and basal cell carcinoma. The result of the present study corresponds well with that of previous studies; squamous cell carcinoma was observed the most (64.5%), followed by basal cell carcinoma (25.8%).

The current problem of CEAC is that there is no consensus on treatment guideline and the optimal management still remains a topic of debate. To our knowledge, no randomized controlled study has been published regarding CEAC owing to its rarity [1]. Consequently, centers that provided data for this study all had different treatment guidelines for CEAC, resulting in different surgical modality and various doses of adjuvant therapy even for the same stage CEAC. In the literature, 5-year overall survival is reported to be 80%–100% for early-stage CEAC and 7%–85% for advanced-stage CEAC [1-5, 8, 18-20]. Because of the relatively low overall survival rate, the standard treatment for CEAC has been lateral temporal bone resection with or without adjuvant RT for the early stage and subtotal temporal bone resection with adju-
vant RT for the advanced stage. Although earlier studies have asserted a necessity for an extensive surgical procedure for CEAC even at an early stage [2, 6, 21], CEAC is no exception to the recent trend of choosing a less aggressive treatment modality for malignant tumors in the head and neck area. Latest studies on CEAC demonstrated that only RT without surgical treatment shows good prognostic results in early-stage patients [22, 23]. Although earlier studies indicated better 5-year survival rate of patients with CEAC who underwent surgical treatment with adjuvant RT compared to those who underwent either surgery or RT, a recent meta-analysis suggested that there is no significant difference in survival between patients who underwent surgery alone and those who underwent adjuvant RT [14, 24]. Therefore, the role of adjuvant RT in survival benefit remains unclear, and to what extent and level of intensity should RT be used is a question that needs to be answered [3, 2, 4, 7-10, 18, 22, 23, 25]. In this study, stage I patients showed 100% survival rate and 0% recurrence rate regardless of surgical type and adjuvant RT. On the other hand, 3 of 7 stage II patients (42.8%) showed recurrence after the initial surgery. Surgical modalities varied in stage-II patients: 3 patients underwent local resection, 3 patients underwent lateral temporal bone resection, and 1 patient underwent subtotal parotidectomy. Surprisingly, all recurrent stage-II patients were treated by different surgical methods, and all received adjuvant RT. No correlation was found between other clinical characteristics and recurrence pattern among stage-II patients. Furthermore, even though all advanced-stage patients received adjuvant therapy of either RT or CRT, survival rate was much poorer than that for early stage (53.6% Vs. 100%). From these results, it can be concluded that the good prognosis of stage I CEAC may propose for less aggressive treatment modality and that local canal resection without adjuvant therapy may be enough for the complete eradication of disease. However, more aggressive surgical management with intensive adjuvant RT should be considered for CEAC of stage II or more to avoid recurrence and subsequently lower the mortality rate.

Another complex factor for choosing the optimal extent of surgery for CEAC is whether or not to include parotidectomy and neck dissection in addition to the resection of main tumor. Because lymph node involvement is a known indicator of poor prognosis, it is generally accepted that neck dissection is needed for clinically node-positive cases [6]. Prophylactic neck dissection may be advisable even for node-negative cases because there is a report that the rate of micrometastasis in clinically negative necks is up to 17% in CEAC [17]. In terms of conducting simultaneous parotidectomy for CEAC, various studies asserted the need for parotidectomy, as the malignant tumor of temporal bone may not only invade parotid directly, but also involve intraparotideal lymph nodes via the fissures of Santorini and glenoid fossa of the temporomandibular joint [11, 26]. Similarly, a study that analyzed medical records of 72 patients with temporal bone cancer found that 36% had direct tumor invasion into the parotid and 25% had secondary invasion through metastatic intraparotid lymph nodes [27]. In our study, 15 of 31 patients (48.3%) received simultaneous parotidectomy. An indication of prophylactic parotidectomy in carcinoma of the EAC is not yet established, 5 of 22 early-stage patients (22.7%) underwent simultaneous prophylactic parotidectomy at the surgeon’s discretion. However, only 2 patients showed pathologically confirmed direct invasion to the parotid, and no intra-parotid lymph node metastasis was found. As for 7 clinically N0 patients who had prophylactic simultaneous neck dissection, only 1 patient showed micro-metastasis of 1 level II lymph node postoperatively. Overall, simultaneous neck dissection showed a trend toward a better survival rate (p=.053), but parotidectomy did not seem to influence the survival (p=.127). Of 3 recurred stage-II patients, 2 patients did not receive neck dissection or parotidectomy. Although this study cannot measure the benefits and risks of routine neck dissection and/or parotidectomy as a limitation of retrospective assessment, this result suggests that CEAC of stage II or more requires such prophylactic surgical treatments.

There are some limitations in the present study. First, this retrospective study can only delineate outcomes of already-treated patients and cannot fully assess the suitability of treatment modality that was decided by initial Pittsburgh stage. In addition, each center that provided data for this study had different treatment guidelines that were largely dependent on the surgeons treating the patients with CEAC. On the other hand, this study has concluded that stage I tumors behave differently from stage II tumors, regardless of various treatments; therefore, the fact that patients were treated in different ways rather strengthened this final statement. A standard treatment modality should be set up first in a future prospective study to confirm the validity of this study. Second, due to the rarity of disease, this study did not differentiate patients by their histologic types. The clinical characteristics and responses to the treatment may vary depending on histopathological types of the carcinoma. Nonetheless, it is clear that this study explicitly showed different prognosis for early-stage and advanced-stage CEAC, regardless of its specific histologic types. Third, although this study suggests that stage I CEAC shows different clinical features and should be treated differently compared with stage-II CEAC, the sample size of stage-II CEAC is small and incongruent to compare. To overcome these limitations, a case-control study with large sample is essential.

CONCLUSION

This study evaluated the treatment results of multicenter case series of CEAC. The results of this study suggests that more aggressive treatment modality, including adjuvant therapy, is necessary for patients with CEAC with Pittsburgh stage II or more. On the other hand, CEAC of stage I may warrant a good prognosis and less invasive treatment modality could be considered.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Catholic Medical Center (IRB No. XC16RIM0043).

Informed Consent: Informed consent is not necessary due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.


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Conflict of Interest: The authors have no conflict of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES


INTRODUCTION
Acute otitis externa (AOE) is the most common infection of the external auditory canal (EAC). High temperatures, humidity, trauma, absence of cerumen, excessive sweating, alkaline pH, and the use of a hearing aid are all risk factors in the development of AOE [1]. Although AOE is primarily a local disease, it may be more severe and invasive in cases where the patients' immune system is suppressed. Edema and sensitivity in the EAC are noted in otoscopic examinations [2].

The most frequently isolated microorganism in cases of AOE is Pseudomonas aeruginosa, which has gram-negative properties and reproduces easily on a moist base [3]. Topical treatment is generally used in the treatment of AOE. When the infected area can be reached by topical drops, systemic antibiotics are not required [4]. Aminoglycosides, polymixin B, quinolones, and acetic acid are generally used as topical antimicrobial agents. The anti-inflammatory effects of these agents reduce edema and pain and can be used alone or combined with corticosteroids [4-6].

Thymoquinone (TQ) is an active constituent isolated from the Nigella sativa plant [7]. Previous studies have shown that the biological activity of Nigella sativa, which is used in traditional medicine, originates from the high ratio of TQ in the content [7]. Since it was first isolated, studies have shown anti-inflammatory, antioxidant, and anticarcinogenic activity [7-8]. The aim of this experimental study was to compare the dose-related effect of TQ with other topical agents on AOE created in a rat model.

OBJECTIVE: The aim of this experimental study was to compare the dose-related effect of topical thymoquinone (TQ) with other topical agents used in the management of acute otitis externa (AOE) in a rat model.

MATERIALS and METHODS: Forty-eight male Wistar albino rats were divided into six groups each with eight rats per group. Group I was the control group with no external otitis, whereas external otitis were created in the other five groups (study groups). Dexamethasone, 0.1% TQ, 0.4% TQ, ciprofloxacin, and 0.9% saline (NaCl) drops was applied once daily in Groups II-VI, respectively. The treatment was administered regularly for 10 days. Pathologic and microbiologic evaluation were performed. Pathologically, the thicknesses of the stroma and the epithelium in the external auditory canal (EAC) were measured using an occlometer. Edema in the stroma, density of inflammatory cells and blood vessels, presence of fibroblasts, and changes in collagen fibers in the EAC were evaluated in five different areas to obtain the area of highest concentration and classified into four grades (0=no change, 1=mild, 2=moderate, 3=severe).

RESULTS: The higher concentration of TQ (0.4%) was more effective than dexamethasone and 0.1% TQ with respect to antibacterial and the anti-inflammatory properties.

CONCLUSION: TQ, particularly at a concentration of 0.4%, may be considered for topical application alone in the treatment of AOE, without any requirement for a combined treatment.

KEYWORDS: Thymoquinone, acute otitis externa, treatment, experimental study

INTRODUCTION
Acute otitis externa (AOE) is the most common infection of the external auditory canal (EAC). High temperatures, humidity, trauma, absence of cerumen, excessive sweating, alkaline pH, and the use of a hearing aid are all risk factors in the development of AOE [1]. Although AOE is primarily a local disease, it may be more severe and invasive in cases where the patients’ immune system is suppressed. Edema and sensitivity in the EAC are noted in otoscopic examinations [2].

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MATERIALS and METHODS

Study Population and Animals
The approval for the study was granted by the Experimental Animal Research Ethics Committee of Mustafa Kemal University. The study was conducted in accordance with the items of the Helsinki Declaration relevant to experimental studies. All the animals were transported to the Experimental Animal Research laboratory of Mustafa Kemal University and were kept in cages with standard conditions and nutrition.

The study included a total of 48 Wistar albino rats, aged 12-16 weeks, weighing 300-400 g, with a healthy EAC, tympanic membrane, and middle ear bilaterally confirmed by an otomicroscopic examination.

External Otitis Model
To create the external otitis model, firstly, the rats were intraperitoneally administered anesthesia of 0.1 mL (90 mg/kg) ketamine hydrochloride and 0.2 mL (10 mg/kg) xylazine. Then, under microscopic guidance, both EACs were traumatized with a plastic micropipette at 80 rotations/minute for 5 minutes. Approximately 1 minute after the trauma, 0.1 mL P. aeruginosa (1.5×10⁵ colony-forming units (CFU)/mL) drops were administered. The standard strain of P. aeruginosa ATCC 27853 was used [9]. The external otitis model was applied to 40 of the 48 rats in the study. After 24 hours, a microscopic view confirmed the development of external otitis in both ears of the 40 rats. Reproduction of the bacteria in the samples taken from the infected ears was also observed [10].

Smear samples were taken for ear cultures at 24 hours after inoculation and on Days 4, 7, and 10 of the treatment. The samples were inoculated in blood agar and eosin methylene blue agar. The colonies produced at the end of 18-24 hours incubation at 37°C were identified using conventional methods. Exclusion criteria for the external otitis groups of rats were defined as the death of an animal during the experiment or no visualization of findings of otitis under the otomicroscopic view.

The rats were randomly separated into 6 groups each with eight rats. A solution of TQ was prepared at concentrations of 0.1% and 0.4% in saline solution. Group I was the control group with no external otitis and no treatment applied. In Group II, 0.1 mL (1 mg/mL) dexamethasone drops were applied once daily to the ears. In Group III, 0.1 mL (1 mg/mL) 0.1% TQ drops were applied once daily. In Group IV, 0.1 mL (4 mg/mL) 0.4% TQ drops were applied once daily. In Group V, 0.1 mL (3.5 mg/mL) ciprofloxacin drops were applied once daily. In Group VI, 0.1 mL 0.9% saline (NaCl) drops were applied once daily. In the total 40 rats in Groups II-VI, where external otitis was created, the treatment was administered regularly for 10 days. No rat from any group died during the study period.

Pathologic Evaluation
Following completion of the treatment, ketamine and xylazine anesthesia was administered intraperitoneally and the rats were euthanized through cardiac exsanguinations. Both temporal bones were excised including the EAC and tympanic membrane and placed in 10% buffered formaldehyde solution. After fixation for 24 hours, the samples were decalcified for 5 days in a decalcification solution (formic acid 98%-100%). After the decalcification procedure, transverse and longitudinal sections were taken to show full layer of the EAC and placed on cassettes; after passing through different degrees of alcohol and xylene solutions, the sections were embedded in paraffin blocks. Sections of 4 μm in thickness were cut, stained with hematoxylin and eosin, and evaluated in a random order by a pathologist blinded to the groups using an Olympus BX53 microscope (ZA 3262, U-OCMC, 24 mm diameter, 10/100X); the modified Emgard et al. [9, 10] classification was used. The thicknesses of the stroma, keratin layer, and epithelium in the EAC were measured using an ocularometer. Edema in the stroma, density of inflammatory cells, density of the blood vessels, presence of fibroblasts, and changes in collagen fibers in the EAC were evaluated in five different areas to give the area of highest concentration, and the results were evaluated using four grades (0=no change, 1=mild, 2=moderate, and 3=severe).

Statistical Analysis
The Statistical Package for Social Sciences version 21.0 (IBM Corp.; Armonk, NY, USA) software was used for statistical analyses. The defined numerical values were compared statistically. For the multiple group comparisons of epithelial and stroma thicknesses measured in the EAC, one-way ANOVA analysis was applied, and in the paired comparisons, the post-hoc Tukey test was applied. A p value of <0.05 was accepted as statistically significant for all the statistical data.

The statistical analysis of the parameters edema, inflammatory cells, fibroblasts, blood vessels, and collagen and the results of the cultures taken on Days 1, 4, 7, and 10 of the study were analyzed using Chi-square analysis and paired comparisons were made for significant parameters.

RESULTS
The mean epithelial and stroma thicknesses of the groups are shown in Figure 1. No statistically significant difference was determined between Groups IV and V with respect to EAC epithelial and stroma thicknesses (p=0.867).

The EAC epithelial and stroma thicknesses of the rats in Groups IV and V were determined to be lower compared to Group VI with statistically significant differences (p=0.009, p=0.008, p<0.001, p=0.004, respectively, Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Dexamethasone+Otitis</th>
<th>0.1% Thymoquinone+Otitis</th>
<th>Ciprofloxacin+Otitis</th>
<th>Saline+Otitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma thickness</td>
<td></td>
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</tbody>
</table>

Figure 1. Mean epithelial and stroma thicknesses of the groups.
When the groups were compared with respect to edema occurring in the EAC stroma, the rates of edema in Groups III, IV, and V were lower compared to Group VI with statistically significant differences (p=0.018, p<0.001, p<0.001, respectively, Table 1). In the comparison of the rates of inflammatory cells between the groups, no statistically significant difference was determined between Groups II, III, and IV. The rates of inflammatory cells in Groups IV and V were determined to be lower compared to Group VI with statistically significant differences (p=0.009, p<0.001, respectively, Table 1; Figure 2).

The rates of fibroblast distribution, extent of vascular infiltration, and presence and distribution of collagen in the groups were compared separately between the groups. The values of the control group were lower compared to those of all the other groups with statistically significant differences, and no significant difference was determined in the other groups with statistically significant differences (Figure 3). The spread of blood vessels was determined to be low in Group V compared to Groups II, III, and VI with statistically significant differences (p=0.017, p=0.001, p=0.007, respectively; Table 2).

In the evaluation of the culture results on Day 1 of the treatment, with the exception of the control group (Group I), reproduction was determined in all the ears in which the external otitis model was created.

When the results of the cultures taken on Days 4, 7, and 10 of the treatment were compared between the groups, the rate of culture positivity on Day 4 in Group V was determined to be lower than those in Groups II, III, IV, and VI with statistically significant differences. The rate of culture positivity on Day 7 in Group V was determined to be lower than those in Groups II, III, and VI with statistically significant differences (p<0.001, p<0.001, p<0.001, respectively) and similar to that of Group IV (p=0.101).

The rate of culture positivity on Day 10 in Groups IV and V was determined to be lower than those in Groups II, III, and VI with statistically significant differences. The culture positivity rates of Groups IV and Group V were similar. No statistically significant difference was determined between the culture positivity rates of Group III and Groups II and VI (p=0.273, p=0.273, respectively). The positivity rates of the culture results on Days 1, 4, 7, and 10 are detailed in Table 3.

DISCUSSION
The results of the current study showed that the thickness of the epithelial cells, stromal thickness, and the rates of inflammatory cells were lower in the Groups IV and V compared to Group VI with statistically significant differences. This indicates that a 0.4% dose of TQ has a strong anti-inflammatory effect.
Table 2. Comparison between the groups of FB, BV, and CL

<table>
<thead>
<tr>
<th>Groups</th>
<th>FB</th>
<th>BV</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-II (Control - Dexa)</td>
<td>p=0.001</td>
<td>p&lt;0.001</td>
<td>p=0.006</td>
</tr>
<tr>
<td>I-III (Control - 0.1% TQ)</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.001</td>
</tr>
<tr>
<td>I-IV (Control - 0.4% TQ)</td>
<td>p&lt;0.001</td>
<td>p=0.002</td>
<td>p=0.002</td>
</tr>
<tr>
<td>I-V (Control - Cip)</td>
<td>p&lt;0.001</td>
<td>p=0.043</td>
<td>p=0.002</td>
</tr>
<tr>
<td>I-VI (Control - Saline)</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.001</td>
</tr>
<tr>
<td>II-III (Dexa - 0.1% TQ)</td>
<td>p=0.796</td>
<td>p=0.764</td>
<td>p=0.790</td>
</tr>
<tr>
<td>II-IV (Dexa - 0.4% TQ)</td>
<td>p=0.583</td>
<td>p=0.196</td>
<td>p=0.776</td>
</tr>
<tr>
<td>II-V (Dexa - Cip)</td>
<td>p=0.583</td>
<td>p=0.017</td>
<td>p=0.776</td>
</tr>
<tr>
<td>II-VI (Dexa - Saline)</td>
<td>p=0.317</td>
<td>p=0.498</td>
<td>p=0.417</td>
</tr>
<tr>
<td>III-IV (0.1% TQ + 0.4% TQ)</td>
<td>p=0.746</td>
<td>p=0.062</td>
<td>p=0.531</td>
</tr>
<tr>
<td>III-V (0.1% TQ - Cip)</td>
<td>p=0.746</td>
<td>p=0.001</td>
<td>p=0.531</td>
</tr>
<tr>
<td>III-VI (0.1% TQ - Saline)</td>
<td>p=0.159</td>
<td>p=0.599</td>
<td>p=0.551</td>
</tr>
<tr>
<td>IV-V (0.4% TQ - Cip)</td>
<td>p=1.000</td>
<td>p=0.210</td>
<td>p=1.000</td>
</tr>
<tr>
<td>IV-VI (0.4% TQ - Saline)</td>
<td>p=0.073</td>
<td>p=0.070</td>
<td>p=0.201</td>
</tr>
<tr>
<td>V-VI (Cip - Saline)</td>
<td>p=0.073</td>
<td>p=0.007</td>
<td>p=0.201</td>
</tr>
</tbody>
</table>

Dexa: dexamethasone; TQ: thymoquinone; Cip: ciprofloxacin; FB: fibroblast distribution; BV: blood vessels; CL: collagen presence and distribution.

In a rat model study by Emgard et al. [9], 100% efficacy of budesonide was determined on Days 10 and 20 of treatment with respect to bacterial eradication. Budesonide alone was stated to have cured the experimental AOE more effectively than a weak steroid combined with an antibiotic hydrocortisone acetate combined with oxytetracycline and polymixin B (HCPB), and this was attributed to the fact that inflammation is a major mechanism in the development of AOE, irrespective of the presence of either bacteria or fungi. However, it was emphasized that the inflammatory reaction of the EAC skin in this animal model of AOE cannot be directly extrapolated to the human situation.

In the current study, bacterial eradication of the topical steroid was determined as 18.75% and 25% on Days 7 and 10 of the treatment, respectively. Similar rates of bacteria eradication were determined in the group administered with saline. In our study, the bacteria eradication rates of the group administered with topical steroid were lower than those reported in the Emgard et al. [9] study with the use of budesonide, which may be explained by topical dexamethasone not exhibiting as potent anti-inflammatory property as budesonide and that the content did not have isopropanol with which the environment developed an acidic pH. In the current study, the similar rates of culture positivity obtained in Groups II and VI can be attributed to the steroid showing a similar effect to that of saline.

In the current study, as the culture positivity of Group V was lower than that of Groups II, III, IV, and VI on Day 4 of treatment and lower than that of Groups II, III, and VI on Days 7 and 10 of treatment both with statistical significance and as a similarity was determined with Group IV in terms of bacterial eradication on Days 7 and 10 in particular, this indicates that a dosage of 0.4% TQ has an antibacterial and anti-inflammatory effect as strong as that of ciprofloxacin. The stronger antibacterial and anti-inflammatory properties of the 0.4% dose of TQ than that of dexamethasone and 0.1% TQ suggest that the antibacterial and anti-inflammatory effect of TQ is dose-dependent.

In a study by Pistorius et al. [11] of 239 patients diagnosed with acute external otitis and treated with 0.2% ciprofloxacin for 7 days, the bacterial eradication rate was 92%. In the same study, 236 patients with AOE were treated with 0.2% ciprofloxacin+0.1% hydrocortisone for 7 days, and the bacteria eradication rate was 95%. Drehobl et al. [12] treated 319 external otitis patients with 0.2% ciprofloxacin for 7 days and 87.5% bacterial eradication was determined for P. aeruginosa. In the evaluation of previous studies, ciprofloxacin topical solution has been shown to have high eradication rates, such as 83.3%-95.7%, against P. aeruginosa [13]. In the ciprofloxacin-treated group in the current study, reproduction was determined in all the smear samples taken on Day 1 of the treatment, and the bacteria eradication rates were found to be 81.25%, 100%, and 100% on Days 4, 7, and 10 respectively. The bacteria eradication rates obtained in this study with the treatment of topical ciprofloxacin for a period of 10 days were consistent with those reported in literature.

Various studies have shown that TQ has anti-inflammatory, antibacterial, antiviral, antiallergic, antioxidant, analgesic, immunomodulator, and anticancer activity [7, 8, 14-18]. In the current study of the external otitis model, that inflammation was lower in the TQ group than that in the saline group with statistically significant difference, and although not significant, the epithelial and stroma thicknesses were lower in the TQ group than those in the dexamethasone group, suggest that TQ could be a promising molecule in the treatment of AOE.

In the current study, that the bacteria eradication rates of the 0.4% TQ on Days 7 and 10 were higher than those of the saline, dexamethasone, and 0.1% TQ groups with statistically significant difference shows that the anti-inflammatory and antibacterial properties were more effective than other topical treatments. The antibacterial effic-
cy of 0.4% TQ is also supported by the finding of antibacterial properties similar to those of ciprofloxacin from the seventh day onwards.

CONCLUSION

In conclusion, the results of the current study showed that in terms of bacteria eradication and the anti-inflammatory property, 0.4% TQ was more effective than dexamethasone and 0.1% TQ, thereby indicating that the anti-inflammatory and antibacterial effects of TQ are dose-dependent. In addition to the antibacterial and anti-inflammatory effects of TQ, as various studies have determined analgesic and antihistaminic properties, it is thought that TQ, particularly at a concentration of 0.4%, could be used topically alone in the treatment of AOE, without any requirement of a combined treatment (17). However, there is a need for further clinical studies to confirm these findings.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Mustafa Kemal University (Approval No: 2014-11/4).

Informed Consent: Informed consent was obtained from the patient who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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The Predictability Precision of Superior Semicircular Canal Through Radiological Assessment and Microanatomical Dissection

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INTRODUCTION
Skull base is highly a complex anatomical area containing important neural and vascular structures. Successful surgery needs appropriate technology, surgical experience, thorough anatomical knowledge, and preoperative radiological evaluation [1]. Therefore, landmarks play a crucial role for reaching the targeted area precisely. Arcuate eminence (AE) and great superficial petrosal nerve (GSPN) are important landmarks for identifying the internal auditory canal (IAC) in middle fossa, extended middle fossa, and transpetrosal–transcentorial approaches [2]. AE, which can be described as the arched prominence on the superior surface of the petrous part of the temporal bone, is suggested as a landmark to navigate the position of the superior semicircular canal (SSC) [2]. Although AE is shown to exist in 100% of all neonates, this proportion is reported as 70% in the adult population. This eminence is more often seen on the left side compared with right and reported to be invisible approximately in 25% of cases [3].

There is still an ongoing controversy for precisely locating SSC by AE. High variability between these two anatomic structures is reported by various studies in the related literature [3-6]. Radiological or microanatomical dissection studies are performed to better delineate the relationship of AE and the SSC [6, 7]. To the best of our knowledge, there is a dearth of investigations consisting of both radiological and anatomical assessments. The present study aims to combine the radiological and microanatomical assessments to further contribute to the relationship of AE and SSC for achieving more acceptable surgical success.

MATERIALS and METHODS
In this study, we used 12 dry skulls that belong to Mersin University Medical Faculty Department of Anatomy. The most prominent part of the temporal bone, which was estimated as the AE was marked with copper wire and fixed with scotch tape. Then, CT assessment was performed with 0.5-mm-thin sections temporal bone algorithm, and the raw data were reformatted with vitrea 2.0 at workstation. The relationship between the AE and SSC was shown by using a 64-slice CT scanner (Aquillion 64, Toshiba Medical Systems Tokyo, Japan).

The distances of the determined three points including: lateral (A), apical (B), medial (C) of the SSC and fixated copper wire were measured radiologically (Figure 1). The distance (H) is the height between the most apical part of the SSC to the surface of the skull base (Figure 2) and it is tried to be measured using the most appropriate vision through lateral oblique and coronal sections. In case an intersection occurred between SSC and copper wire, the angle between them was calculated (E) (Figure 3).

Ethics committee approval was received for this study from Mersin University Clinical Research Ethical Committee.

Statistical Analysis
The minimum and maximum values and mean and standard deviation values from the descriptive statistics for the angle and distance measurements were given. The number and percentage values from the descriptive statistics for categorical variables were also given. The normal distributive control was done by Shapiro-Wilk test for the angle and distance values. The difference between right-left angle and right distance-left distance measurements were evaluated by paired t test among parametric tests. Direct variant analysis method (ANOVA) was used for the investigation of angle and diameters between each other found in the right and the left. Statistical significance value was considered as (p) 0.05 for all statistical comparisons.
Dissection
We drew a line with a pen in accordance with the copper wire. A high-speed drill (Bien Air- Suisse) and operation microscope (C.Z.; OPMI pico, Germany) were used for the dissection of SSC using AE. The distances were measured with electronic caliper (Figure 4), and the angles were measured with a copper wire and goniometer (Figure 5).

The angles between SSC and the midpoint of the IAC (F) (Figure 6) and SSC to the sulcus of the GSPN (G) (Figure 7) were measured. The nearest distance was measured between the most posterior part of the SSC and the point marked by the perpendicular line drawn from the medial border of the petrous bone to the most posterior part of the IAC (D) (Figure 8).

RESULTS
The mean values of radiological evaluation parameters (A, B, C, and H distances) regarding right–left comparison are given in Table 1. The values and the comparison of right-left E angles are given in Table 2.

The same anatomic evaluation parameters regarding the comparison of the same side are given in Table 3. The total number of intersections are given in Table 4 showing the relationship of the copper wire and the SSC. Table 5 shows the mean values of F, G, and D. The attached copper wire was found to be intersected to SSC in three cas-
Table 1. The right and left comparison of A, B, C and H distances

<table>
<thead>
<tr>
<th></th>
<th>MIN-MAX</th>
<th>MEAN±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right-A</td>
<td>0.01-9.40</td>
<td>2.549-2.756</td>
<td>0.73</td>
</tr>
<tr>
<td>Left-A</td>
<td>0.01-6.85</td>
<td>2.922-2.40</td>
<td></td>
</tr>
<tr>
<td>Right-B</td>
<td>0.01-11</td>
<td>3.667-3.158</td>
<td>0.972</td>
</tr>
<tr>
<td>Left-B</td>
<td>0.01-8.50</td>
<td>3.627-2.935</td>
<td></td>
</tr>
<tr>
<td>Right-C</td>
<td>0.01-12.50</td>
<td>5.855-3.769</td>
<td>0.871</td>
</tr>
<tr>
<td>Left-C</td>
<td>1.10-11.80</td>
<td>6.087-3.401</td>
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</tr>
<tr>
<td>Right-H</td>
<td>0.60-2.80</td>
<td>1.325-0.655</td>
<td>0.783</td>
</tr>
<tr>
<td>Left-H</td>
<td>0.40-2.20</td>
<td>1.266-0.540</td>
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</tr>
</tbody>
</table>

Table 2. The right and left comparison of E angles

<table>
<thead>
<tr>
<th></th>
<th>MIN-MAX</th>
<th>MEAN±SD</th>
<th>p</th>
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<tr>
<td>Right-E</td>
<td>13.80-65.20</td>
<td>30.313-12.84</td>
<td>0.369</td>
</tr>
<tr>
<td>Left-E</td>
<td>4.40-61.85</td>
<td>35.558-18.44</td>
<td>0.369</td>
</tr>
</tbody>
</table>

Table 3. The same anatomic evaluation parameters regarding the comparison of the same side

<table>
<thead>
<tr>
<th></th>
<th>MIN-MAX</th>
<th>MEAN ± SD†</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIGHT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>0.01-9.40</td>
<td>2.549-2.756</td>
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<tr>
<td>B</td>
<td>12</td>
<td>0.01-11</td>
<td>3.667-3.158</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>0.01-12.50</td>
<td>5.855-3.769</td>
</tr>
<tr>
<td>LEFT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>0.01-6.85</td>
<td>2.922-2.240</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>0.01-8.5</td>
<td>3.627-2.935</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>1.10-11.80</td>
<td>6.087-3.401</td>
</tr>
</tbody>
</table>

Table 4. The relationship between the copper wire and the SSC

<table>
<thead>
<tr>
<th></th>
<th>Right</th>
<th>Left</th>
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</thead>
<tbody>
<tr>
<td>Nearly Overlap</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Intersected</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Posterolateral</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

SSC: superior semicircular canal

Table 5. The mean values of right-left F, G, and D

<table>
<thead>
<tr>
<th></th>
<th>MIN-MAX</th>
<th>MEAN ± SD†</th>
<th>p</th>
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<tr>
<td>Right-F</td>
<td>37.00-90.00</td>
<td>53.17-17.83</td>
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<td>28.00-80.00</td>
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<td>Right-G</td>
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<td>100.92-15.54</td>
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<td>4.36-10.59</td>
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<td>0.76</td>
</tr>
<tr>
<td>Left-D</td>
<td>5.48-10.67</td>
<td>8.13-1.80</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The right and left comparison of E angles

Discussion

There are three well-defined methods to localize the IAC in middle fossa surgeries [1]. The House Method consists of tracing the GSPN to find the facial nerve and the fundus of IAC [2]. Garcia-Ibanez method aims to drill between GSPN and the AE by bisecting the area between these two structures [2].

Fisch method explains blue lining the SSC and localizing the IAC using a fairly constant angle 45-60 degrees [2]. The angle found in our study was 47 degrees for the left side and 53 degrees for the right side. Our measurement was performed between SSC and midpoint of IAC. Statistical significance was not found between right and left angles. The average angle was reported as 61 degrees by Rhoton in their microsurgical anatomical study.

Wigand technique finds the IAC by bisecting the angle between GSPN and SSC instead of AE [8]. This angle shown by the present study may help the surgeon drill the roof of the IAC, if the dissection is decided to be performed between these two structures. The distance between the cortical petrous bone and the apex of the SSC is another important point to deal with. This depth and the presence of air cells between the SSC and the floor of the temporal bone is of great importance during drilling of this area for the preservation of hearing in middle fossa surgeries (Figure 9 a, b). The authors want to emphasize upon the technique of measurement when one wants to compare the angles. Therefore, our findings were concordant with the related literature with the mean angle of 52 degrees in range of 34-75 degrees. The mean angle between the sulcus of the GSPN and

es for the right and five for the left. Posterolateral location of the wire to the SSC was found in seven cases in the right and six cases in the left. Meanwhile, the wire near overlaps was observed in two cases in the right and just one in the left.
SSC is measured as 93.58 in the right and 100.92 in the left. A similar measurement was performed by Maina et al. [9] between GSPN and AE reported as 122.92 in the range of 96.29-158.90.

The apex of the SSC to the floor of the temporal bone was 1.33 mm for the right and 1.27 for the left side. This distance was given as 2 mm by Katsuta et al. [7] with a range of 0.2 mm to 4.2 mm [8].

Correspondence between the AE and SSC was also examined by CT evaluation. The distances between the SSC and the most prominent part of the petrous part of the temporal bone, which is thought as the AE, were measured at three positions including medial, lateral, and apical points. Statistical significance was not found between the right and left values of A, B, and C distances. On the other hand, distance A was found to be significantly greater when compared to the other two distances. This fact points out that the predictability of the SSC by AE diminishes toward the midline. These data might indicate to the skull base surgeons a greater caution toward the medial border of the petrous ridge to prevent sensorineural hearing loss due to SSC drilling. In the present study, a great majority of the attached copper wires (13 of 24) was found to be localized posterolateral to the SSC. Seo et al. [3] reported lateral location as 25/52 in their series [2]. High variability of AE with respect to SSC is documented by very low percentages of complete correspondence of these two anatomic structures. In our study, near overlap was found in two cases for the right whereas just one for the left side. Seo et al. [3] reported complete correspondence as 2/52 [3].

Therefore, preoperative CT imaging and/or navigation systems are to be cautiously evaluated to find out the precise relationship between AE and SSC for successful middle fossa approaches and prevent any important complications including hear loss.

CONCLUSION

Various structures had been reported to play the role on the formation of AE other than SSC. Elevation or depression on the cerebral surface cannot be excluded for supporting the formation of AE by the gyrus or a sulcus on the inferior aspect of temporal lobe [10]. On behalf of that majority of cases, AE did not correspond with SSC and temporo-occipital sulci participation is blamed in its formation with an inconstant fashion [5, 11]. This is also emphasized in Djallilian’s et al. [12] reporte that the angle was 42.3 degrees between SSC and the posterior wall of the IAC, whereas this angle was 60.8 degrees when measured between SSC and anterior wall of the IAC.

The hypotheses mentioned above are to be studied with greater number of dry skulls and cadavers to better enlighten the precise relationship of the aforementioned anatomical structures. MRI and CT scanning of the temporal bone and mentioned structures may give valuable information.

REFERENCES

Evaluation of Benign Paroxysmal Positional Vertigo in American Football Players

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Department of Otolaryngology, Head and Neck Surgery, Koç University Hospital, Istanbul, Turkey

OBJECTIVES: The aim of this investigation was to evaluate the association between posterior channel benign paroxysmal positional vertigo (BPPV) and trauma that is frequently experienced by American football players.

MATERIALS and METHODS: Participants were classified into the following two groups: (1) a study group consisting of 63 male participants aged 18-30 years who had been playing American football for more than 2 years and (2) a control group consisting of 49 male participants aged 18-27 years with no history of otologic/vestibular disease or acute/chronic trauma. Trauma, age, total duration of playing American football, and weekly training hours of subjects in the study group were analyzed to determine any relationship with BPPV occurrence. We performed otologic, audiologic, and vestibular assessments of pure sound audiometry, tympanometry, tandem walking test with eyes open and eyes closed, Romberg, head shaking, roll, and Dix-Hallpike tests to all participants.

RESULTS: A positive correlation between the total years of American football played and posterior channel BPPV frequency was observed in the study group. In addition, increasing weekly hours of training was shown to further increase the risk of BPPV. A total of 16 out of 63 athletes experienced BPPV, whereas none of the participants in the control group experienced BPPV. All participants completed the Vertigo Symptom Scale, which revealed that vertigo did not cause any significant negative impact on their training routine and activities of daily living.

CONCLUSION: Our results indicate that the weekly training hours and total years of training with American football increase posterior channel BPPV frequency.

KEYWORDS: American football, benign paroxysmal positional vertigo, sports, trauma, vertigo
Written informed consent has been obtained from all participants. The physical examination methods and diagnostic tests were explained to all participants. Before undergoing the tests, all participants completed the Vertigo Symptom Scale that represented the effects of vertigo on the activities of daily living based on a scale from 0 to 4 [7].

Participants
Volunteers were divided into the following two groups: a study group with athletes and a control group. There were 63 male athletes aged between 18 and 30 years in the study group. The control group consisted of 49 male individuals aged between 18 and 27 years, who had no complaints regarding vertigo or dizziness during the evaluation, no clinical history of peripheral and/or central vestibular system disease, and no history of acute and/or chronic trauma. Individuals with peripheral and/or central vestibular system disease, otologic disease, and conductive or sensorineural-type hearing loss were observed in their pure sound audiometric examination. The exclusion criteria included any history of surgery and ages under 18 or over 30 years.

Clinical Analysis
We performed otologic examinations, pure sound audiometric test (Interacoustics AC40 Clinical Audiometer, Denmark), Dix–Hallpike maneuver with videonystagmography (VNG) goggles, Romberg test, tandem walking test with eyes open and eyes closed, and VNG (Micro Medical, USA) on all patients.

All positional tests were performed, and their results were recorded. Dix-Hallpike maneuver was performed using VNG goggles. All individuals were rapidly brought from sitting to a supine position, with the head turned 45° to one side and extended approximately 30° backward. Once supine, the eyes were typically observed for approximately 30 seconds. If no nystagmus was present, the person was brought back to a sitting position. After a 30-second pause, the other side was tested. Dix–Hallpike test was evaluated as positive if brief latency is observed between the onset of nystagmus and change of the head position and manifestation of a paroxysmal upbeat and torsional nystagmus accompanied with a sense of vertigo.

Pure sound audiometric tests were performed for measuring hearing sensitivity. Air and bone conduction thresholds were determined between 125-8000 Hz and 500-4000 Hz sound stimuli, respectively. Pure tone average is calculated at 500, 1000, and 2000 Hz frequencies for each participant.

Statistical Analysis
All data obtained in this study were interpreted using Statistical Package for Social Sciences version 20.0 (IBM Corp.; Armonk, NY, USA) statistical program. Kolmogorov-Smirnov and Shapiro-Wilk tests were used for evaluating normalization. Comparing tests between the groups were performed with Mann-Whitney U, Fisher’s exact, and chi-squared tests. Statistical significance of variables in the study was assessed using logistic regression analysis.

RESULTS
The average age was 22.86±0.41 years for the American football athletes and 22.37±0.41 years for the control group. There was no statistically significant difference noted between the control and study groups according to age (p>0.05). Table 1 shows the average age, weekly training hours, and total years of practicing football for each group.

The median score of the Vertigo Symptom Scale was 1.21 out of 4 for the control group and 1.33 out of 4 for the study group.

Results of the audiologic examination showed that pure sound average scores were 12 dB for 8, 8 dB for 27, 7 dB for 30, 5 dB for 22, and 3 dB for 25 of the participants. All participants had type A tympanogram. Results of Romberg, tandem walking, head shaking, and roll tests were negative for all individuals. All VNG results did not indicate any vestibular pathology.

Dix–Hallpike test was negative for all participants in the control group; however, it was positive in 16 out of 63 (25.4%) athletes in the study group. Overall, 9 of the athletes had left posterior canal BPPV, and 7 of them had right posterior canal BPPV. There was a statistically significant difference noted between the two groups (p<0.001).

Logistic regression analysis was used for determining the relationship between BPPV and weekly training hours and total years of practicing. Table 2 and Figure 1 present the results. When Beta scores for variables were examined, training hours and total years of practicing...
American football showed a positive correlation with BPPV occurrence. According to the calculations of odds ratio, the weekly training hours and total years of practicing American football are defined as risk factors for BPPV.

**DISCUSSION**

Vertigo is defined as a hallucination of motion, which can be a result of any pathology in the vestibular system [2]. BPPV is a vestibular system disease manifesting as sudden dizziness triggered by certain positions of the head and is accompanied with peripheral nystagmus. Epley et al. [8] reported that BPPV comprises 25% of all vertigo etiologies. Even though BPPV is perceived as an extremely irritating and frightening condition by the patient, it can be effectively cured with simple physical maneuvers or can even spontaneously resolve.

BPPV is triggered by head movements and is mostly idiopathic. However, its etiology includes head and temporal bone traumas, stapedectomy, chronic otitis media surgery, vestibular neuritis, Ménière’s disease, migraine, hypertension, long bed rest, upper respiratory tract infections, and long-distance airway or overland travels. In literature, from 8.5% to 20% of all BPPV cases are associated with traumatic causes. Gordon et al. [22] investigated 247 patient records with posterior canal BPPV and emphasized that minor head traumas and short and severe neck and head traumas during MVA cause traumatic BPPV. Patients with a history of MVA were treated with physical therapy, and 57% relapsed intermittently compared with those with idiopathic BPPV (p<0.04) [23].

Suarez et al. [23] assessed the significance of the age and gender of patients with light head trauma. The study on 325 patients with idiopathic BPPV and 51 with traumatic BPPV reported that traumatic BPPV is more common in the young population compared with idiopathic BPPV, presents generally bilaterally, and shows no gender difference unlike idiopathic BPPV [23].

In the retrospective analysis of 716 traumatic BPPV cases, 23.4% of BPPV cases were claimed to have been caused by trauma [24]. In our study, 16 out of 63 (25.4%) American football athletes showed positive results in Dix-Hallpike test, whereas in the control group, there were no positive test results. Similar to other hypotheses regarding the relationship between trauma and BPPV, we claim that head trauma leads to displacement of otoconia into SCC. As American football players are exposed to head trauma, they experience posterior channel BPPV. Liu et al. [25] outlined that 55% of patients with traumatic BPPV and 6.5% of patients with idiopathic BPPV experience single or double canal BPPV symptoms (p<0.01) following the comparison between 40 patients with traumatic BPPV and 46 patients with idiopathic BPPV. Bilateral SCC affected 25% of the traumatic BPPV cases [23]. Patients with BPPV were treated with a single Epley maneuver; however, 67% of the traumatic BPPV and 12% of the idiopathic BPPV had relapsed at 1 year follow-up (p<0.001) [23].

Ahn Seong-Ki et al. [26] reported a relationship between traumatic brain injury and BPPV. They described clinical characteristics of BPPV after traumatic brain injury and compared idiopathic BPPV cases between 2003 and 2009 [26]. Kerr et al. [17] stated that treating traumatic BPPV is more challenging and has a higher relapse risk than idiopathic BPPV (p<0.05). Gordon et al. [10] noted that only 2 out of 21 patients with post-traumatic BPPV have horizontal canal involvement. In our study, all of the patients with BPPV showed posterior canal involvement.

**CONCLUSION**

Numerous studies indicate that BPPV is associated with the practice of certain sports such as boxing, swimming, and mountain bike riding. To the best of our knowledge, a relationship of BPPV with American football has not been studied before our study showed a positive correlation between the two. Previous studies did not examine the relationship between frequency of exercise and BPPV. Our study...
shows that weekly hours of training and number of years spent practicing are both risk factors for posterior channel BPPV in American football athletes.

The primary limitation of our study is the limited number of athletes who are recruited from only one team in Turkish Universities American Football League. In future research studies, we aim to obtain larger sample sizes for the study and control groups and try to identify the mechanisms that could induce BPPV in the incidents of trauma.

Ethics Committee Approval: Ethics committee approval was received for this study from Turgut Özal University School of Medicine Ethics Committee for Clinical Research (Approval Date: 20.02.2015/Approval No: 2015/03).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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Oxidative Status in Patients with Benign Paroxysmal Positional Vertigo

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Department of Otolaryngology, Head and Neck Surgery, Ümraniye Training and Research Hospital, İstanbul, Turkey (ID)
Department of Otolaryngology, İstanbul Okmeydani Training and Research Hospital, İstanbul, Turkey (MED)
Department of Medical Biochemistry, Dişkapı Yıldırım Beyazıt Training and Resarch Hospital, Ankara, Turkey (CB, ÖE)

OBJECTIVE: Benign paroxysmal positional vertigo (BPPV) is the most frequent peripheral vestibular disorder and is particularly seen among older patients suffering from vertigo. The brief vertigo attacks in and imbalance symptoms of BPPV are caused by freely floating otoconia within the semicircular canals. The aim of this prospective study was to evaluate the role of oxidative stress, using native thiol/disulfide (SH/SS) homeostasis as a novel indicator, in the etiology of BPPV.

MATERIALS and METHODS: The 62 participants in the study included 31 patients with BPPV and, as the control group, 31 healthy individuals without any cochleovestibular disorders.

RESULTS: Patients with BPPV initially had significantly lower native SH levels and significantly lower SH/total thiol (TT) ratios, as well as significantly higher SS/SH and SS/TT ratios, than the healthy controls. After successful treatment of their vertigo, which was confirmed based on the results obtained from the second blood sample, patients with BPPV still had lower SH levels and SH/TT ratios and significantly higher SS/SH and SS/TT ratios than the healthy controls.

CONCLUSION: Our results suggest a role of oxidative stress in the development of BPPV, through both calcium metabolism and the direct toxic effects of free oxygen radicals, including the triggering of apoptosis.

KEYWORDS: Benign paroxysmal positional vertigo, oxidative stress, thiol, disulfide

INTRODUCTION

Benign paroxysmal positional vertigo (BPPV) is the most frequent peripheral vestibular disorder; it is particularly seen among older patients suffering from vertigo [1]. Brief vertigo attacks in and imbalance symptomatic of BPPV are caused by otoconia freely floating within the semicircular canals [2]. Vertigo occurs after specific head movements and has the characteristics of nystagmus, with respect to latency time, fatigability, and transiency. Canalithiasis and cupulolithiasis are the most likely mechanisms underlying BPPV. Although any of the three semicircular canals may be involved, canalithiasis of the posterior semicircular canal is the underlying cause in at least 85% of patients [3-5].

Oxidative stress, which is defined as excessive production of reactive oxygen species (ROS) that is not counterbalanced by adequate endogenous and exogenous antioxidant defenses, causes cellular dysfunction and is a risk factor for microvascular injury [6]. Several studies have shown an elevation in oxidative stress levels in different pathologies, with higher-than-control levels of biomarkers such as modified lipids, proteins, and nucleic acids, reduction in antioxidant capacity, and increased ROS production by leukocytes. In otolaryngology, the relationship between oxidative stress and laryngeal cancer, hearing loss, rhinosinusitis, otitis media, chronic tonsillitis, and other conditions has been investigated [7-10]. In a recent study, it has been revealed that calcium metabolism and its relationship with oxidative stress may play a role in the development of BPPV [11].
The aim of this prospective study was to evaluate the role of oxidative stress, using native thiol thiol/disulfide (SH/SS) homeostasis as a novel indicator, in the etiology of BPPV.

MATERIALS and METHODS

Patients and Controls
This prospective two-center study was conducted from June 2017 to July 2017 at the Department of Otolaryngology Head and Neck Surgery of the Ümraniye Training and Research Hospital and at the Department of Otolaryngology Head and Neck Surgery of İçerenköy Hospital, Bayındır Health Care group. The study was approved by the Ethics Committee of Ethics Committee of Ümraniye Training and Research Hospital (number 2017/76). Written informed consent was obtained from all participants recruited for the study.

Totally, there were 62 participants: 31 patients with BPPV (study group) and 31 healthy individuals without any cochleovestibular disorders (control group).

Patients with hearing loss, a history of otologic surgery, neurologic disorders, smoking, malignancy, autoimmune disorders, hypertension, endocrine disorders including diabetes mellitus and hypothyroidism, cardiovascular disease, a history of antiaggregant therapy, infectious diseases, or other inflammatory conditions were excluded.

Vestibular and Acoustic Evaluation
All participants underwent a complete otorhinolaryngologic examination, including a neuro-otologic examination. The otologic examination included otomicroscopy to visualize the tympanic membrane for vesicles attributable to herpes zoster infection and to determine the presence of chronic ear disease or retraction pockets with cholesteatoma. The Valsalva maneuver and pushing on the tragus cartilage (fistula test) were performed; it was evaluated whether either would induce a vertigo attack, which would have suggested a perilymphatic fistula. All participants underwent pure-tone audiometry. The neurologic examination focused on gait, balance, and coordination. Gait and balance were assessed using Romberg’s sign and the Fukuda stepping test. The presence of cerebellar signs was evaluated to exclude central pathologies.

For the diagnosis of BPPV in patients whose symptoms worsened with sudden head movements, videonystagmography-assisted Dix–Hallpike and supine roll tests (Pagnini–McClure maneuver) were performed. The characteristic pattern of BPPV nystagmus and the history of the disease were recorded for all patients in the study group. Only those with posterior canal BPPV were included. Patients in the study group had geotropic, torsional nystagmus beating toward the undermost ear. The nystagmus duration was less than 60 s, with a latency of a few seconds and a decline in response upon performing repeat maneuvers. Epley’s repositioning maneuver was performed in the treatment of patients with BPPV. The Dix–Hallpike test was repeated for all patients with BPPV 2 days after the diagnosis of posterior canal BPPV; Epley’s repositioning maneuver was repeated if patients complained of vertigo, nausea, or vomiting or if nystagmus was determined during the Dix–Hallpike test. The latter test was performed in control individuals 21 days after the first hospital admission.

Blood Sample Collection
Peripheral venous blood samples were obtained from the patients with BPPV upon first hospital admission (during the vertigo attack) and 21 days after treatment. Blood samples were obtained from the healthy controls during a routine medical examination. Plain tubes were used to collect blood from the patients and controls. The samples were centrifuged for 10 min at 1,500 g, after which the serum was separated and stored at -80°C until further analysis. Serum SH, total thiol (TT), and SS levels were analyzed in these samples, and SS/SH, SS/TT, and SS/TT ratios were calculated according to the method of Erel and Neselioglu.\(^{[11]}\)

Statistical Analysis
The NCSS 2007 program (NCSS, Kaysville, Utah, USA) was used for statistically evaluating the data. The mean, standard deviation, median, minimum, maximum, frequency, and percentage values served as descriptive statistics. Normally distributed data were compared between the groups with the independent samples t-test. The paired samples t-test was used for within-group analysis of normally distributed data, and Pearson’s chi-squared test was applied to evaluate qualitative data. A p-value of <0.05 was considered to indicate statistical significance. The 95% confidence intervals were also determined.

RESULTS
There was no statistically significant difference between the study and control groups with respect to the mean age (59.65±10.97 and 58.35±4.75 years, respectively) or male:female ratio (14:17 and 14:17, respectively) (Table 1). The demographics of the patients with BPPV and healthy controls are shown in Table 1.

However, the differences between the oxidative parameters in the patient and control groups were statistically significant. At baseline, the patients with BPPV had significantly lower SH levels and SH/TT ratios and significantly higher SS/SH and SS/TT ratios than the healthy controls. After treatment for vertigo, the patients with BPPV still had lower SH levels and SH/TT ratios and significantly higher SS/SH and SS/TT ratios than the healthy controls (Figure 1, 2) (Table 2).

Table 1. Demographic parameters

<table>
<thead>
<tr>
<th></th>
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<th>Patient group (n=31)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>Min–Max</td>
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<td>51–67</td>
<td>34–78</td>
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<tr>
<td></td>
<td>Mean±SD</td>
<td>59.00±8.41</td>
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<td>59.65±10.97</td>
</tr>
<tr>
<td>Gender; n (%)</td>
<td>Female</td>
<td>34 (54.8)</td>
<td>17 (54.8)</td>
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<tr>
<td></td>
<td>Male</td>
<td>28 (45.2)</td>
<td>14 (45.2)</td>
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</tr>
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</table>

1 Independent samples t-test
2 Pearson's chi-squared test
SD: Standard deviation
## DISCUSSION

To the best of our knowledge, this is the first study to investigate SH/SS homeostasis as a novel marker of oxidative stress in patients with BPPV. Specifically, we examined SH/SS homeostasis in a study group of patients with BPPV and a control group of healthy individuals. Our study showed a significant difference between the groups.

Free oxygen radicals are naturally generated during every reaction in the body. Normally, these unstable electron-laden chemicals are largely destroyed or removed by the body’s natural antioxidant defense systems. Oxidative stress occurs due to an inadequate response to the formation of free radicals. Among the major non-enzymatic antioxidants able to eliminate oxidative stress in the cell are sulfhydryl group (-SH) containing SHs [11]. Circulating blood albumin binds SH groups via albumin cysteine residues. Reversible SS bonds form with cysteine residues located at the active sites of the protein, thereby reducing the toxicity of ROS [12, 13]. TT levels in cells remain constant to ensure continuous SH/SS homeostasis, reflecting the turnover between SSs and SHs [11].

A global plasma oxidative stress index was recently developed and validated in several diseases. Oxidative stress is related to cardiovascular diseases and their risk factors, such as diabetes, hypertension, and obesity, all of which are highly prevalent in numerous countries all over the world. A relationship between a decline in thiol levels and several systemic diseases has been demonstrated in many studies [14, 15]. While SH/SS homeostasis could previously only be measured by measuring the levels of individual components, Erel and Neselioglu [11] described a new method that allows for the measurement of the levels of these compounds both individually and cumulatively.

Episodes of dizziness are common in the elderly and significantly increase the risk of falls. The incidence of BPPV increases with age. Peripheral vestibular dysfunction, including BPPV, is one of the most common causes of dizziness among the elderly and is one of the most frequent diseases seen in dizziness clinics. The results of our study revealed that oxidative stress may be one of the etiologic factors in the development of BPPV. However, in our study, while SH and TH levels were significantly lower in the study group than in the control group, SS levels did not significantly differ between the groups. Patients with BPPV were treated with Epley’s repositioning maneuver and then followed up (outpatient visits) until nystagmus was resolved. SH and TH levels increased after treatment, but this was not statistically significant. The SS/SH, SS/TT, and SH/TT ratios, which represent corrected values and predominantly indicate ox-

### Table 2. Serum native thiol/disulfide and total thiol levels

<table>
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<tr>
<th></th>
<th>Treatment</th>
<th>Admission</th>
<th>p&lt;sub&gt;1&lt;/sub&gt;</th>
<th>p&lt;sub&gt;2&lt;/sub&gt;</th>
<th>p&lt;sub&gt;3&lt;/sub&gt;</th>
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</thead>
<tbody>
<tr>
<td><strong>SH</strong></td>
<td></td>
<td>280.30-454.80</td>
<td>128.60-395.30</td>
<td>218.90-394.20</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td>375.95±38.95</td>
<td>305.22±59.70</td>
<td>308.60±45.08</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td><strong>TT</strong></td>
<td></td>
<td>329.80-514.50</td>
<td>162.27-456.51</td>
<td>248.68-451.32</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td>417.98±40.92</td>
<td>347.03±66.03</td>
<td>352.88±47.90</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td><strong>SS</strong></td>
<td></td>
<td>15.70-34.45</td>
<td>1.10-38.71</td>
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<td>0.956</td>
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<tr>
<td>Mean±SD</td>
<td></td>
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<td>20.91±9.77</td>
<td>22.14±7.96</td>
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<td><strong>SS/SH</strong></td>
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<td>3.52-22.32</td>
<td>0.671</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td>5.76±1.72</td>
<td>10.23±4.81</td>
<td>10.67±4.45</td>
<td>0.630</td>
</tr>
<tr>
<td><strong>SS/TT</strong></td>
<td></td>
<td>3.72-8.87</td>
<td>0.44-13.75</td>
<td>3.29-15.43</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td>5.12±1.32</td>
<td>8.23±3.42</td>
<td>8.59±2.91</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td><strong>SH/TT %</strong></td>
<td></td>
<td>82.26-92.56</td>
<td>72.49-99.11</td>
<td>69.14-93.42</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td>89.75±2.65</td>
<td>83.54±6.84</td>
<td>82.83±5.83</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

*Independent samples t-test

*Pair samples t-test

**p<0.01

SD: standard deviation; SH: native thiol; TT: total thiol; SS: disulfide

### Figure 1. Native thiol levels

### Figure 2. Total thiol levels
Iwasaki et al. [22] showed a correlation between age-related decrease in oxidative stress markers in blood samples from patients with BPPV. However, there was no significant difference in oxidative stress parameters in the patients with BPPV before treatment versus after treatment. Although oxidative stress may play a role in the development of BPPV, the increase in oxidative stress did not respond to the treatments that we administered to our patients. Güçlütürk et al. [16] studied the levels of the antioxidant paraoxonase in patients with BPPV before and after treatment and reported results similar to ours.

In the semicircular canals, the presence of otocional debris originating from the utriculus and sacculus causes BPPV when the head position is changed quickly and suddenly [2]. Otoconia consist of calcite, a mixture of calcium and carbonates. The structure of otoconia differs from that of teeth and bones because of its carbonate, rather than phosphate, composition. In the inner ear, calcium and carbonate levels are under the control of the calcium channel transport system. Normal levels of calcium and carbonate are important for the maintenance of otocional function. Vibert et al. [17] designed an osteoporosis model in ovariectomized rats and studied the ultrastructure of their otoconia. The otoconia of these rats were less dense and contained less calcium; similar mechanisms may underlie the pathogenesis of BPPV. Talaat et al. [18] found that patients with recurrent or non-recurrent BPPV had significantly lower levels of vitamin D than control patients, while patients with recurrent BPPV had significantly lower vitamin D levels than those with non-recurrent BPPV. In another study, Talaat et al. [19] found a relationship between the recovery of serum 25-hydroxyvitamin D3 levels and a significant reduction in the rate of BPPV relapse.

Oxidative stress is related to calcium metabolism, with the endoplasmic reticulum being the most important cellular site for calcium storage and protein folding. In the presence of cell stress, the endoplasmic reticulum may initiate an increase in cellular calcium levels, causing rupture of the mitochondrial membrane and apoptosis.

Recent studies have suggested that oxidative stress and inner ear diseases are related. Brosel et al. [20] reported a strong link between oxidative stress, the related apoptosis of cochlear cells, and age-related hearing loss. Dinc et al. [21] found significant differences in SH/SS homeostasis between patients with sudden sensorineural hearing loss and control patients. Tsai et al. [21] reported increased levels of oxidative stress markers in blood samples from patients with BPPV.

Iwasaki et al. [22] showed a correlation between age-related decrease in vestibular function and age-related decline in vestibular hair cells and neurons. The underlying mechanism of age-related cell loss in the vestibular end organ is not known, but the cumulative effect of a genetic predisposition and oxidative stress may play an important role. They recommended conducting further studies on the protective effect of antioxidant therapies with respect to vestibular function during aging.

These studies, together with the present results, indicate a role of oxidative stress in the development of BPPV, through both calcium metabolism and the direct toxic effects of free oxygen radicals, which trigger apoptosis. These mechanisms may also have a synergistic effect.

CONCLUSION
Our study was conducted on a small patient group. Further studies with larger samples are needed to evaluate and compare other oxidative stress markers, such as paraoxonase and arylesterase, as well as the total antioxidant status in patients with BPPV.

Ethics Committee Approval: Ethic committee approval was received for this study from Ethics Committee of Umranie Training and Research Hospital (Decision No: 2017/76).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES


The Reliability and Validity of “Dokuz Eylül University Meniere’s Disease Disability Scale”

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OBJECTIVE: Ménière’s Disease (MD) is a chronic, non-life threatening inner ear disease, with attacks of disabling vertigo, progressive hearing loss, and tinnitus as the major symptoms. All three symptoms, separately or in combination, cause great distress and have a considerable impact on the quality of life of the patients. The aims of this study were to develop a disease-specific quality of life survey for patients with MD and to analyze the relationships between the audiovestibular findings and the survey.

MATERIALS and METHODS: Following Ear-Nose-Throat examination and audiovestibular tests, the Dokuz Eylül University Meniere’s Disease Disability Scale (DEU-MDDS) and Turkish version of the Dizziness Handicap Inventory (DHI-T) were administered to 93 patients with definite MD. Reliability and validity analyses of the scale were performed.

RESULTS: There were 45 (48.4%) male and 48 (51.6%) female patients and the mean age was 48.9±12.1 years. Cronbach's alpha was 0.92 and intraclass correlation coefficients of the DEU-MMDS were significant (p<0.001). Results of the Goodness of Fit Statistics showed that the expression levels of the items were high and the correlation coefficients of each item with the scale were sufficient. There was a statistically significant correlation between DHI-T scores and MDDS. DEU-MDDS was not related to the vestibular tests, age or gender (p>0.05).

CONCLUSION: The MDDS is a valid and reliable scale as a disease-specific quality of life questionnaire for patients with MD.

KEYWORDS: Meniere's disease, vertigo, quality of life, hearing loss

INTRODUCTION

Meniere’s disease (MD) is an idiopathic syndrome characterized by endolymphatic hydrops. Vertigo attacks are accompanied by hearing loss, tinnitus, and fullness in the pathological ear [1-3]. Vertigo is the major symptoms and their effect on balance function is a key concern for patients, which may affect their daily functions negatively. Although MD is not regarded as life-threatening, most patients consider their condition as life-altering. The symptom complex can have a dramatic influence on a patient’s quality of life [4, 5]. Quality of life (QoL) can be described as the subjective value placed on one's satisfaction with their life. It encompasses the patient's subjective perception of health, psychological status, social interactions, physical state, and functional abilities [6]. Studies regarding the use of QoL in identifying diseases, staging patients, and assessing the success of treatments found a rapidly increase in the recent years [5, 7, 8]. Since 1972, the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) has published three versions of the recommended guidelines for reporting results of MD treatment. In the last revision, a six-point functional level scale was added whereby the patients assess the effect of vertigo on their daily activities [9]. This scale can be considered as the first tool to evaluate QoL of the patients with MD. In recent years the use of QoL scales in MD patients has increased [9]. However, hearing loss, tinnitus, imbalance, and QoL were evaluated by different scales in most of these studies and general or field-specific scales were not specific to MD [10]. MD differs from other otological conditions in terms of complaints about the hearing loss and vertigo attacks. Attack features and inter-episode conditions are also specific to the disease and the patient. Therefore, patients must be evaluated individually with a specially...
developed scale for MD. Disease-specific QoL scoring systems are very effective methods for also assessing a patient’s perceived experience of a particular disease [7].

There are two MD-specific QoL surveys in the literature. The first one is the Meniere’s Disease-Patient Oriented Severity Index (MD-POSI) [6, 14]. The other survey is the Meniere’s Disease Outcome Questionnaire (PDOQ), which was generated by Kato et al. [12] in 2004. Neither has been widely used in the literature. Their structural validities have not been analyzed yet.

Based on this information, the first aim of this study was to develop an original QoL scale for MD patients. The other purpose was to evaluate the relationship between the survey and the audiovestibular features of the patients.

MATERIALS and METHODS

Between June 2014 and March 2015, 93 patients diagnosed as having definite MD according to the 1995 AAO-HNS criteria were included in the study conducted by our department of Otolaryngology-Head and Neck Surgery, Hearing-Speech and Balance Unit. After a detailed medical and otological history, including clinical and familial characteristics, all patients underwent a detailed otological examination followed by audiovestibular investigations. The audiological tests were pure tone and speech audiometry, as well as acoustic immittance measurements. Pure tone and speech audiotests were performed using an Interacoustics AC-40 device (Interacoustics A/S, Denmark), which is a two-channel audiometer in a double wall and a double suites audiometry booth. For audiometric results, Goodman’s classification was accepted as the reference [13]. Acoustic immittance measurements were done using an Interacoustics AZ-7 device (Interacoustics A/S, Denmark) and the findings were analyzed according to Jongkees’ classification [14].

Videonystagmographic (VNG) evaluation, bithermal caloric test, positional tests, and other tests such as head-shaking, clinical head impulse, Romberg’s and sharpened Romberg’s, Unterberger’s stepping, and eyes open/closed tandem gait tests were performed. VNG evaluations were done with Vortex equipment (Visual Eyes Binocular goggles, FireWire 100 Hz, Eyemax Spectrum Balance Software: Micromedical Technologies, IL, USA). The test protocol included saccadic, tracking and optokinetic eye movement evaluations, and recordings of gaze and spontaneous nystagmus, as well as head-shaking nystagmus, bithermal caloric, and positional tests. For the bithermal caloric test, the maximum slow-phase velocity of nystagmus was calculated after each irrigation, and canal paresis and directional preponderance were determined according to Jongkees’ formula. If the asymmetry between the responses for the left and right ears was X 21%, the result was considered to be indicative of significant canal paresis. For directional preponderance, a difference between the right and left beating nystagmus of X 28% was considered pathological. The caloric test was considered normal when both (canal paresis and directional preponderance) were within normal limits. Following audiovestibular assessments, the Dokuz Eylül University Meniere’s Disease Disability Scale (DEU-MDDS) and the Turkish version of the Dizziness Handicap Inventory (DHI-T) were administered by an audiologist [15, 16].

The Dizziness Handicap Inventory (DHI) is the most widely used scale to assess the self-perceived handicapping effects imposed by vestibular system diseases. The patient answers “yes”, “sometimes” or “no” to each question and the strength of the responses are designated with numeric values of 0, 2, and 4. The questionnaire has 25 items, such that the total score ranges from 0 to 100, with a higher score indicating a higher handicap [15].

The originally-developed DEU-MDDS, is an MD-specific QoL scale inspired by the characteristics, clinical course, and other features of MD, as well as a careful review of other scales developed previously for MD, along with other neuro-otological diseases. Since MD is a disease with acute disabling vertigo episodes (spells, attacks) and inter-episodic imbalance periods without attacks, those features needed to be assessed separately. For that reason, the scale consists of two factors; there are subscales for “acute episode” and “between the episodes,” with 52 questions for each. The acute episode subscale includes 13 items about physical symptoms during attacks and includes 13 items. The between the episodes subscale includes 39 items assessing daily and self-care activities, restrictions on participation in social life and employment. The questionnaire was completed during patient interviews with the supervision of an audiologist. Each answer was taken on a scale between 1 and 5 (1: never and 5: always) according to the Likert scale technique [17]. Higher scores indicated a higher disability. Each sub-section score and the overall total score of the scale were calculated. Results of the survey were first calculated as a score and then the disability as a percent (Figure 1).

Exclusion criteria from the study were non-volunteering, a presence of an additional central nervous system pathology, an age under 18 or over 70, or a presence of congenital nystagmus or any other diseases that could lead to dysconjugate eye movements.

All numeric, ordinal, and nominal data were analyzed by using Statistical Package for Social Sciences version 20.0 (IBM Corp.; Armonk, NY, USA) and LISREL 8.8 (Latent Structural Relation Scientific Software International Inc, IL, USA) statistics softwares. The descriptive statistics (frequencies for nominal and ordinal values; means and standard deviations for scale values), correlation coefficients (Spearman’s test), t-test, reliability tests (Cronbach’s alpha, model fitting ANOVA, Tukey’s Additivity test, Hotelling’s T-square statistics, intraclass correlation coefficients, item-total correlation coefficients, corrected item-total correlation coefficients and Cronbach’s alpha if item-deleted), face and content validities, exploratory factorial analyses (Varimax rotation with Kaiser normalization) and confirmatory factorial analyses (Goodness of Fit Statistics) were also completed. Face and content validities were measured by consulting with ten experts. The expert panel consisted of 3 otorhinolaryngologists, 5 audiologists (PhD), 1 occupational therapist (PhD), and 1 psychologist (MSc). Face validity is concerned with how appropriate, relevant, and clear the items on a questionnaire are concerning the aim of the scale. In order to assess content validity, the content validity ratio (CVR) and content validity index (CVI) were calculated. For calculating CVR, the expert panel was requested to comment independently on the necessity of each item using a 3-point Likert scale; 1=essential, 2=useful but not essential, and 3=unessential. Following the expert’s assessments, a CVR for the total scale was computed. According to Lawshe’s Minimum Value Table, an accept-
able CVR value for 10-expert panels is 0.62 or above [18]. For the CVI, the same expert panel was asked to evaluate the individual items (1=not relevant, 2=somewhat relevant, 3=quite relevant, and 4=highly relevant) on “relevancy,” “clarity,” and “simplicity” [19, 20]. CVI scores of DEU-MDDS were calculated by determining the proportion scores of 3 or 4 by all experts.

Reliability analyses are used to evaluate the reliability of instruments used for measurement. The basic assumption of the reliability analysis is that each question is a linear component of the total score. There must be an additivity feature in the scale. Tukey’s Additivity test was performed to assess the additivity feature of DEU-MDDS. Whether the question averages are equal to each other were tested using Hotelling’s T-square statistics.

For the test-retest reliability of the DEU-MDDS, a subsample of definite MD patients (n=20) completed the scale twice with a two-day interval in order to examine the stability of the DEU-MDDS by calculating intraclass correlation coefficients.

This study was approved by the local ethical committee (2014/22-41). All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all patients.

RESULTS
Forty-five (48.4%) patients were male, 48 (51.6%) were female and the mean age was 48.9±12.1 years. The mean duration of MD was 5.6±4.7 (min: 10 months; max: 14 years) years. Forty-two cases were pathological in the right ear (n=42 patients, 45.2%), and in the left ear (n=45 patients, 48.4%). There were six (6.5%) bilateral cases. The mean attack time was 5.2±9.8 h. 40.9% of patients had one or more accompanying chronic diseases. The most common comorbidities were hypertension (8.6%), coronary artery disease (5.4%), thyroid related pathologies (5.4%), depression (3.2%), and hypertension plus diabetes mellitus (3.2%). The familial MD history was 8.6%. The description of at least one attack trigger was 77%; the highest values were stress (35.5%), stress plus seasonal changes (9.7%), seasonal changes only (5.4%), stress plus effort (4.3%), and stress plus sleepiness (3.2%). The audiological findings regarding patients with unilateral and bilateral MD are shown in Table 1. Degrees of hearing loss in the pathological ears were mild in 36.8%, moderate in 32.2%, moderately severe in 19.5%, severe in 9.2%, and profound in 2.3% of unilateral MD patients. Type A and As tympanograms were obtained in 89.7% of patients and acoustic reflexes were obtained in 78.2% of involved ears of unilateral cases. All of the bilateral MD cases had Type A and As tympanogram and acoustic reflexes were positive in 66.7%.

Gaze evoked nystagmus was not observed in any of the patients. Spontaneous nystagmus was recorded in 15 patients (16.1%). Head-shaking nystagmus was detected in 19 patients (20.4%). Pathological finding ratios of VNG tests were 1.1% for saccadic, 8.6% for tracking, and 14% for optokinetic eye movements. Findings of the Table 1. Pure tone and speech audiometry means and standard deviations of the patients with unilateral and bilateral Meniere’s Disease (MD)

<table>
<thead>
<tr>
<th></th>
<th>Unilateral MD n=87</th>
<th>Bilateral MD n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathological ear</td>
<td>Healthy ear</td>
<td>Right ear</td>
</tr>
<tr>
<td>Means of 0.5-2 kHz air conduction thresholds (dB HL)</td>
<td>46.8±21.4</td>
<td>15.6±13.9</td>
</tr>
<tr>
<td>Means of 0.5-3 kHz air conduction thresholds (dB HL)</td>
<td>47.4±22.3</td>
<td>18.8±15.1</td>
</tr>
<tr>
<td>Speech discrimination scores (%)</td>
<td>71.5±23.6</td>
<td>93.7±6.3</td>
</tr>
</tbody>
</table>

Table 2. Dizziness Handicap Inventory-Turkish version mean scores and standard deviations

<table>
<thead>
<tr>
<th></th>
<th>Mean Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical subscore (9 items)</td>
<td>15.93±8.91</td>
</tr>
<tr>
<td>Emotional subscore (7 items)</td>
<td>7.3±4.78</td>
</tr>
<tr>
<td>Functional subscore (9 items)</td>
<td>16.62±8.85</td>
</tr>
<tr>
<td>Total (25 items)</td>
<td>38.8±19.5</td>
</tr>
</tbody>
</table>

The CVR value was 0.99 and at the acceptable range (higher than 0.62). The CVI value of the DEU-MDDS was also 0.99. I-CVI and S-CVI values were 0.90 and 0.96, respectively. These CVI values were considered to demonstrate acceptable content validity. All 52 items of the DEU-MDDS had a CVI over 0.80; therefore, all items were retained.

The exploratory factorial loadings of DEU-MDDS were analyzed. The extraction method was principal component analysis and the rotation method was Varimax rotation with Kaisers’ normalization. As a result of this analysis, 20 incompatible items (5 from the acute episode subscale and 15 from the between the episodes subscale) to the two-factorial structure were excluded from the scale (factorial loadings of these items were lower than 0.4). Thus, the number of DEU-MDDS items was decreased from 52 to 32. It was noticed that the excluded items had lower corrected item-total correlation coefficients and if item-deleted Cronbach’s alpha values than the others. The new 32-item version of the DEU-MDDS was analyzed by exploratory factorial analysis again; it was shown that the DEU-MDDS had a two-componential factorial loading structure (Table 3).

The confirmatory factorial analyses were performed by the Goodness of Fit Statistics with the 32-item version of the scale. For the confirmatory factor analysis, chi-square (χ²), Root Mean Square Error of approximation (RMSEA), Root Mean Square Residual (RMR), Goodness of Fit Index (GFI), Adjusted Goodness of Fit Index (AGFI) and Comparative Fit Index (CFI) were calculated. For statistical analysis values lower 5, above 0.6 and, being lower than 0.2 of values were
considered acceptable level for $\chi^2$, GFI and AGFI, SRMR and RMSEA respectively for model data fitting [21-24]. The statistics on compliance of confirmatory factor analysis of the DEU-MDDS are given in Table 4. The compliance indexes obtained by confirmatory factor analysis of the structural models related to the DEU-MDDS show that there was a good agreement between the models and the data. The ratio of the chi-square value to the degree of freedom was 1.79, indicating a good compliance between the model and data. The levels of AGFI and GFI were above the 0.60 level and the CFI, NFI, and IFI values were higher than 0.80, also pointing to a sufficient fitting between the model and data. Being lower than 0.9, the SRMR value indicated that the model compatibility related to standardized errors of the model was a sign of the data fitting. It was noted that the RMSEA value covered a value of 0.08 within 90% probability. This also suggested that the model data alignment was sufficient [25]. It could be said that the generated DEU-MDDS model had a sufficient level of conformity with the data and structural validity when all of the model data compliance values for the scale were examined. For the Goodness of Fit Statistics, t-tests and $R^2$ (the model coefficients) calculations were also performed. It could be assumed that the items could measure the DEU-MDDS implicit variables. All t and standard values (chi-square=705.69, the degree of freedom=459, $p<0.001$, RMSEA=0.076) showed significant relations between both the implicit (DEU-MDDS) and the observed variables (each item of DEU-MDDS). These findings indicated that the definition levels of the items to implicit variables were high and the relations of item-scale were sufficient. $R^2$ values were higher than 0.1 except for items 7 and 8. As a result of all these analyses, the scale was simplified and the highest structural validity with the 32-item form was structured (Table 5).

The reliability of the internal consistency of the 32-item DEU-MDDS was measured with four indices; Cronbach’s alpha (0.92), intraclass correlations (0.896, $p=0.0001$), Tukey’s additivity test ($p=0.0001$, $F=67.06$, $a=2.63$, Grand mean=2.571), and Hotelling’s T-square tests ($p=0.0001$, $F=73.25$). These values were deemed indicative of good reliability.

Table 6 shows the 32-item DEU-MDDS scores as means and disability as percent. The acute episode subscale mean score was 33.69±6.96 out of 40 points and the between the episodes subscale mean score was 58.35±21.47 out of 120 points. The total score was 92.06±24.54 out of 160 points.

A group of 20 MD patients (9 male, 11 female) ranging in age from 25 to 69 years (45.75±13.57 years) were administered the scale. Intraclass correlation-coefficients were computed for the total score, acute episode, and between episodes subscales of the 32-item DEU-MDDS. The test-retest reliabilities for the total score ($r=0.899$, df2=19, $p<0.001$), for the acute episode subscale ($r=0.894$, df2=19, $p<0.001$), and for the between the episodes subscale ($r=0.899$, df2=19, $p<0.001$) were good.

There were no relations between DEU-MDDS and age, gender, working status, duration of disease and degree of hearing loss. DEU-MDDS and DHI-T scores were evaluated in relation to each other and a significant relation was found between them (Table 7).

Table 3. Factorial loadings of the 32 item Dokuz Eylül University Meniere’s Disease Disability Scale

<table>
<thead>
<tr>
<th>Rotated Component Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>AE4</td>
</tr>
<tr>
<td>AE8</td>
</tr>
<tr>
<td>AE2</td>
</tr>
<tr>
<td>AE7</td>
</tr>
<tr>
<td>AE3</td>
</tr>
<tr>
<td>AE1</td>
</tr>
<tr>
<td>AE10</td>
</tr>
<tr>
<td>AE13</td>
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<tr>
<td>BE32</td>
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<tr>
<td>BE33</td>
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<tr>
<td>BE34</td>
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<tr>
<td>BE35</td>
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<tr>
<td>BE17</td>
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<tr>
<td>BE19</td>
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<tr>
<td>BE18</td>
</tr>
<tr>
<td>BE14</td>
</tr>
<tr>
<td>BE4</td>
</tr>
<tr>
<td>BE7</td>
</tr>
<tr>
<td>BE23</td>
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<td>BE38</td>
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<tr>
<td>BE5</td>
</tr>
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<td>BE37</td>
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<td>BE1</td>
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<tr>
<td>BE2</td>
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<tr>
<td>BE24</td>
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<td>BE11</td>
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<td>BE13</td>
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<tr>
<td>BE12</td>
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<tr>
<td>BE22</td>
</tr>
<tr>
<td>BE39</td>
</tr>
<tr>
<td>BE3</td>
</tr>
</tbody>
</table>

AE: Items of the acute episode; BE: Items of between the episodes

Table 4. The significance of the Goodness of Fit Statistics and prominent values

<table>
<thead>
<tr>
<th>DEU-MDDS</th>
<th>$\chi^2$</th>
<th>df</th>
<th>NFI</th>
<th>RMSEA</th>
<th>SRMR</th>
<th>GFI</th>
<th>AGFI</th>
<th>CFI</th>
<th>IFI</th>
<th>90%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEU-MDDS: Dokuz Eylül University Meniere’s Disease Disability Scale; $\chi^2$: Minimum Fit Function Chi-Square; df: degrees of freedom; NFI: Normed Fit Index; GFI: Goodness of Fit Index; AGFI: Adjusted Goodness of Fit Index; CFI: Comparative Fit Index; IFI: Incremental Fit Index; SRMR: Standardized Root Mean Square; RMSEA: Residual Root Mean Square Error of Approximation; 90%CI: 90 Percent Confidence Interval for RMSEA</td>
<td>827.7</td>
<td>461</td>
<td>0.84</td>
<td>0.084</td>
<td>0.087</td>
<td>0.66</td>
<td>0.61</td>
<td>0.92</td>
<td>0.92</td>
<td>0.073; 0.094</td>
</tr>
</tbody>
</table>
DISCUSSION

The main objective of the use of disease-specific QoL scales is to determine the effects of the disease on QoL. It is difficult to measure the effects of MD because the severity of the symptoms and the disease characteristics vary over time and from patient to patient. In our clinical practice, we have realized that previously reported vertigo and/or balance related QoL surveys are not completely compatible with MD characteristics. For MD, the questionnaire should be specific not only to the disease but also to the episodes and/or time between the episodes.

The AAO-HNS guide (1995) suggests the use of audiometric findings, number of attacks and the Functional Level Scale (FLS) for reporting the improvement of patients with MD. The FLS is the first example of a QoL measurement for this group. The sensitivity of the FLS to the physical and functional effects of MD is good but it cannot evaluate emotional and/or psychosocial situations [26].

There are two MD-specific QoL surveys in the literature. The first one is the Meniere's Disease-Patient Oriented Severity Index (MD-POSI), which was generated by Murphy MP and Gates G in 1999. In 2005 Gates G and Verall AM simplified and published the second version of the MD-POSI [6, 11]. The survey assesses the symptoms and functional status of MD patients under four sections. Six items contain questions about the disease and treatment outcomes without any scoring. Two questions examine treatment methods. With this scale, no...
Disabilities are not always visible, however. Laboratory tests do not completely reflect the reality. Chronic diseases that cause symptoms such as vertigo or imbalance affect all areas of life and are perceived differently from patient to patient with age, gender and social status among the contributing factors. Therefore, while evaluating a patient clinically the tools must contain some parameters that explore how daily life is affected by the disease.

In this study, DEU-MDDS was administered to 93 definite MD patients with 52 items (13 items for attack period and 39 items for the non-attack period) initially. As a result of the exploratory and structural factorial analysis, the number of items was reduced to 32. Administration of the final version of the scale does not require a large time investment during clinical practice. The questions are well understood, and all of the items show a significant correlation with each other and the scale. The independence of the scale from the age, gender, and working status of patients and the duration of the disease indicates the applicability of the scale to any MD patients. This feature is a “must-have feature” in this type of questionnaire [5, 33-35]. Demographic features, familial MD history, accompanying other systemic chronic disease history, and bilaterality of the disease findings were similar to those of other studies [36-41]. It has been reported that the emotional stress is the most powerful attack indicator [31, 41-44]. Our finding was the same. Moreover, the audiological, eye movement, and bithermal caloric test findings of the patients were similar to the literature [19, 35, 37-39, 45-52]. Head-shaking nystagmus has been reported as 60% previously, though the value was 20.4% in this study [38].

It has been reported that ear fullness, tinnitus, hyperacusis, falling, and motion limitations could affect QoL in MD patients [33]. In another study, it was reported that “vertigo” was the chief symptom and that “hearing loss” and “tinnitus” affect the patient psychosocially [8]. Studies stating the negative emotional effects of MD and the positive effects of increasing coping strategies are apparent in the literature [54-58].

In a study evaluating 181 MD patients, functional effects of the disease, activity and participation restrictions, and environmental and individual factors were examined. The functional effects include emotional and mental functions, sleeping problems, fear of attack, and feelings of powerlessness, shame, and guilt. Activity restrictions include walking (especially in darkness), use of public transport (short or long distance), and driving (especially at night). Participation restrictions are related to social life, work, personal relationships, sports, hobbies, and other social activities. Environmental factors include use of hearing aids, eating habits, alcohol use, and expectations of relatives. Lifestyle, habits, and personality are affected by individual differences. In this group, the most significant factor was a fear of an unpredictable, threatening, frightening, and/or uncontrollable attack in a work or social environment [59].

Another study in eighty-six definite MD patients reported that symptoms could negatively affect the health-related QoL. Vertigo and imbalance cause anxiety, negatively affecting driving and/or work performance, and psychological well-being. Timing of the attacks is unclear. Vertigo, fullness of ear, hearing loss, living alone, having lower work status, and hopelessness were found to be factors related to decreased QoL [59].

### Table 6. Dokuz Eylül University Meniere’s Disease Disability Scale findings of the Meniere’s Disease patients

<table>
<thead>
<tr>
<th>Mean Score±SD</th>
<th>Disability as percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Episode Subscale</td>
<td>33.69±6.96</td>
</tr>
<tr>
<td>Between the Episodes Subscale</td>
<td>58.35±21.47</td>
</tr>
<tr>
<td>Total Score</td>
<td>92.06±24.54</td>
</tr>
</tbody>
</table>

### Table 7. The relations between Dokuz Eylül University Meniere’s Disease Disability Scale (MDDS) and Dizziness Handicap Inventory-Turkish version (DHI-T) scores (disability as percentage)

<table>
<thead>
<tr>
<th>MDDS</th>
<th>DHI-T</th>
<th>r</th>
<th>p</th>
<th>r</th>
<th>p</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Episode Subscale</td>
<td>0.220*</td>
<td>0.034</td>
<td>0.263*</td>
<td>0.011</td>
<td>0.292**</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Between the Episodes Subscale</td>
<td>0.239*</td>
<td>0.021</td>
<td>0.478**</td>
<td>0.0001</td>
<td>0.487**</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Total Score</td>
<td>0.196</td>
<td>0.06</td>
<td>0.39**</td>
<td>0.0001</td>
<td>0.397**</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

(Spearman’s Correlation coefficients; r: correlation coefficients, p: significance)

- **Correlation is significant at the 0.01 level.
- *Correlation is significant at the 0.05 level.

**SUBSCALE SCORES:**

\[
\text{Disability as percent for each subscale} = \left( \frac{\text{Sum of sub-scale scores}}{\text{Maximum possible sub-scale score}} \right) \times 100
\]

**TOTAL SCORE:**

\[
\text{Total disability as percent} = \left( \frac{\text{Sum of all scores}}{\text{Maximum possible total score}} \right) \times 100
\]

**Figure 1.** The percent calculation formulas of sub-scales and total score of DEU-MDDS

DEU-MDDS: Dokuz Eylül University Meniere’s Disease Disability Scale

A single score can be determined since only the first 16 questions used a Likert scale type, and other questions are open-ended. At the same time, questions related to the otologic symptoms, the emotional effects of MD and self-care activity limitations are not sufficient. This scale has been used in some studies evaluating the outcomes of different treatment modalities in MD patients [27, 28]. The other survey is the Meniere’s Disease Outcome Questionnaire (MDOQ), generated by Kato et al. [12] in 2004. This scale was principally developed for patients that had received endolymphatic sac surgery and has also been used to measure outcomes of other treatment methods of MD patients assessing functional, mental and social well-being QoL parameters [26, 29-32]. The MDOQ is restricted to patients in the non-treatment period. Neither the MD-POSI nor the MDOQ has been widely used in the literature. Their structural validities have not been analyzed yet.

In peripheral vestibular disorders, the audiovestibular test battery gives a profusion of information about improvement after treatment.
The most popular survey, the DHI, is a reliable tool to assess patients with vestibular disorders, but not appropriate for the episodic structure of MD [9, 10, 28, 34]. Items in the DHI are grouped with three scales. However, it is reported that the scale’s scoring system might not be sufficiently sensitive to the minor changes and that Likert scales could be more appropriate [27]. For this reason, in this study, a 1 to 5 Likert scale has been chosen as the scoring system for the DEU-MDDS [17]. In a study, the DHI total scores were 22.67 ± 12.55 points in bilateral MD cases and 17.72 ± 9.98 points in unilateral cases [39]. In another study, the DHI total score was 39 ± 21 points [40]. In the present study, the mean total DHI score in unilateral MD patients was 38.8 ± 19.5 points. The significance of the relationship between DEU-MDDS and DHI-T was also evaluated in this study. The correlation coefficients of the between the episode subscale were higher than those of the acute episode scale of the DEU-MDDS. This result is thought to originate from the limited capacity of the DHI to measure the symptoms in the acute stage. Moreover, the relatively low DHI-T scores could be a result of this condition.

CONCLUSION

As a conclusion, age, gender, degrees of hearing loss nor duration have affected the DEU-MDDS scores. There was a significant relationship between DEU-MDDS and DHI-T. As a part of a clinical follow-up tool for patients with MD, the DEU-MDDS is a valid and reliable health-related, disease-specific QoL scale.

Ethics Committee Approval: Ethics committee approval was received for this study from Dokuz Eylül University Non-invasive Researches Ethical Commit tee (2014/22-41).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES


INTRODUCTION
The endolymphatic sac (ES) in the inner ear comprises a part of the membranous labyrinth. The vestibule, semicircular canals, and cochlea also contain membranous labyrinth that is filled with the endolymph. It is thought that the ES absorbs the endolymph and maintains balance in specific ionic components (low K⁺ and high Na⁺) to regulate the volume and hydrostatic pressure of the endolymph [1, 2]. Dysfunction of the ES is considered to induce excess of endolymph, leading to endolymphatic hydrops typically observed as bulging of the Reissner's membrane in the cochlea, and causes vertigo and hearing loss [3].

Tight junctions (TJs) are a junctional complex between epithelial cells sealing the paracellular space across the epithelia. TJs have been observed by the freeze-fracture electron microscopy as the network of intramembranous strands (TJ strands) [4], formed by the assembly of claudins and TJ-associated myelin and lymphocyte protein and related proteins for vesicle trafficking and membrane link (MARVEL) proteins. Bicellular TJs (bTJs) are composed of TJ strands laterally connecting two neighboring cells, whereas tricellular TJ strands are formed by vertically extending TJ strands at the contact point of three cells [4].

The ES epithelia have also been revealed to have the TJs in an electron microscopic study [5]. We previously reported the mRNA expression of several claudins in the ES and suggested that claudins participate in paracellular ion transport to maintain the unique ionic concentration and electrical charge of the endolymph [6].

OBJECTIVES: Tricellulin is a tight junction (TJ)-forming protein that participates in the sealing function of tricellular TJs. Tricellulin-knockout (Tric−/−) mice show progressive hearing loss with degeneration of hair cells in the cochlea without physiological or physical disorders. In the present study, we investigated the tricellulin expression and its deletion effects in the endolymphatic sac (ES) using Tric−/− mice.

MATERIALS and METHODS: The ES epithelia from wild-type (WT) mice were laser-microdissected, and RT-PCR was performed. The ES sections from Tric−/− and WT mice were immunostained with an anti-tricellulin antibody. Hematoxylin and eosin staining was performed for morphological examination. The inner ear of Tric−/− mice was perfused with biotinylation reagents, and the ES sections were observed for tracer permeability assay after applying streptavidin–Alexa Fluor 488 conjugate.

RESULTS: The tricellulin expression was confirmed by RT-PCR and by immunohistochemistry in the WT ES. The ES in Tric−/− mice showed normal morphology and revealed no biotin leakage from the lumen.

CONCLUSION: The ES in Tric−/− mice showed no changes in morphology or disruption in macromolecular barrier function. The effects of solute leakages in the ES of Tric−/− mice may be very limited and compensatable, or that the ES epithelia may have other sealing system covering the lack of tricellulin.

KEYWORDS: Tricellulin, endolymphatic sac, paracellular transport, tight junction, knockout

INTRODUCTION
The endolymphatic sac (ES) in the inner ear comprises a part of the membranous labyrinth. The vestibule, semicircular canals, and cochlea also contain membranous labyrinth that is filled with the endolymph. It is thought that the ES absorbs the endolymph and maintains balance in specific ionic components (low K⁺ and high Na⁺) to regulate the volume and hydrostatic pressure of the endolymph [1, 2]. Dysfunction of the ES is considered to induce excess of endolymph, leading to endolymphatic hydrops typically observed as bulging of the Reissner's membrane in the cochlea, and causes vertigo and hearing loss [3].

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Tricellulin (MARVELD2), a member of the TJ-associated MARVEL protein group, is mainly located at the tTJs, although it also localizes sparsely to the bTJs. The epithelial cells (mouse Eph4 cells) with the *tricellulin* (*Tric*) gene knocked down show disorganized formation and increased ion permeability in both tTJs and bTJs [7]. Therefore, tricellulin is thought to play an important role in the formation and barrier function of tTJs and bTJs. Moreover, permeability to macromolecules in tTJs decreased when *Tric* was overexpressed in Madin–Darby canine kidney (MDCK) II cells independently to the ion permeability [8]. *Tric* is also known as a causative gene for recessive nonsyndromic familial deafness (DFNB49) in humans and mice [9]. We recently investigated *Tric*-knockout (KO; *Tric−/−*) mice, which suffered from early-onset progressive hearing loss associated with the degeneration of hair cells in the cochlea [10].

Despite the reported barrier dysfunction in the *Tric*-deficient epithelial cell lines and the severe cochlear dysfunction in *Tric−/−* mice, we were unable to detect vestibular dysfunction in *Tric−/−* mice [10], suggesting that the function of the endolymphatic system can be maintained even without tricellulin. However, the details of the tricellulin expression and the effects of the tricellulin deletion in the ES have not yet been studied. In the current study, we first confirmed the tricellulin expression in the ES of wild-type (WT) mice and tested whether the morphology and barrier function to macromolecules in the ES are maintained in the absence of the tricellulin expression using *Tric−/−* mice.

**MATERIALS and METHODS**

**Animals and Tissue Preparation**

*Tric−/−* mice (accession no. CD80806K, http://www2.clst.riken.jp/arg/mutant%20mice%20list.html) were used, and its generation was previously described by Kamitani et al. [10]. The ethics committee of the Animal Care and Use Committees of our institutions approved the study.

For hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC), deeply anesthetized mice were perfused with a fixative solution of 70% ethanol/RNase-free water and dehydrated. The temporal bones were removed, immersed in 70% ethanol/10% formalin for approximately 5 min and decapitated. The temporal bones were removed and fixed in 4% PFA in PBS for 6 h at 4°C, followed by decalcification in 0.12 M ethylenediaminetetraacetic acid (EDTA) at 4°C for 7 days. Decalcified temporal bones including the ES were substituted with a graded sucrose series and embedded in optimal cutting temperature (OCT) tissue compound for cryosectioning.

For laser capture microdissection (LCM), deeply anesthetized mice were perfused with a fixative solution of 70% ethanol/RNase-free water and decapitated. The temporal bones were removed, immersed in 70% ethanol/RNase-free water for 6 h at 4°C, and decalcified in 0.12 M EDTA including RNA later (Thermo Fisher Scientific, Waltham, MA, USA) for approximately 5 days at 4°C and then embedded in OCT tissue compound for LCM.

**Laser Capture Microdissection**

LCM was performed as previously described [11]. In brief, the entire ES was sliced (10–15 μm thick) using a cryostat at −20°C, refixed, and dehydrated. The whole ES epithelia were microdissected using the Arcturus PixCell II system (MDS Analytical Technologies, Sunnyvale, CA, USA). The LCM samples were proceeded to extract RNA using the PicoPure RNA Isolation Kit (Thermo Fisher Scientific). One RNA sample (LCM–ES) was extracted from the bilateral ES epithelia samples.

**RT-PCR and Sequencing**

RNA of the ES epithelia isolated from the LCM samples was reverse-transcribed and amplified using the SuperScript® III One-Step RT-PCR System with Platinum® Taq (Thermo Fisher Scientific). The specific primer pairs were the following: *Tric* (accession no. NM_001038602.4) forward: 5′-GCCGGAGGTTGGAAAATCA-3′ and reverse: 5′-GGTACCATCTGGA-CAAGACGTT-3′, amplicon size: 106 bp and *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*; accession no. NM_001289726.1) forward: 5′-GAGAGTTTTTCCCTGTCCC-3′ and reverse: 5′-TGGAACAAATCCTC-CATTGTGC-3′, amplicon size: 119 bp. The primers were designed using Primer-BLAST (NCBI, Bethesda, MD, USA). PCR products were separated and visualized on 1.0% agarose gels by electrophoresis. Positive and negative controls were used, with RNA sample from the kidney of the WT mouse and PCR template replaced with pure water, respectively. Purified PCR products were checked for nucleotide sequences by an Applied Biosystems ABI 3130 Genetic Analyzer (Thermo Fisher Scientific) using BigDye Terminator ver. 3.1 (Thermo Fisher Scientific).

**H&E Staining**

After washing with distilled water, the ES sections (7–10 μm thick) from *Tric−/−* mice were placed in hematoxylin without stirring for 10 min, washed with water at 40°C for 15–20 min, and stained with 0.1%–0.5% eosin for 1–3 min. Samples were dehydrated, permeabilized with xylene, mounted, and observed under a light microscope (Olympus BX51; Olympus, Tokyo, Japan). Cochlea samples were made from *Tric−/−* mice temporal bones on postnatal day 21, paraffin-embedded, and stained with H&E [10].

**Immunohistochemistry**

Immunostaining was performed as previously described [12]. Briefly, sections (7–10 μm thick) were fixed with 4% PFA in PBS and washed, and a blocking reagent (Protein Block Serum-Free; Dako Japan, Tokyo, Japan) was applied. Primary antibodies against tricellulin (diluted at 1:100 in PBS; Afinity™ Recombinant Rabbit Monoclonal Antibody-Purified; Thermo Fisher Scientific) and zonula occludens-1 (ZO-1; diluted at 1:200; TJP1/ZO-1 rat anti-mouse monoclonal antibody; LSBio, Seattle, WA, USA) were reacted to the sections overnight at 4°C. After repeated washes, the sections were incubated with secondary antibody Alexa Fluor 488 donkey anti-rabbit IgG or Alexa Fluor 555 donkey anti-rat IgG (diluted at 1:200 in PBS; Thermo Fisher Scientific) for 2 h at 23°C and washed. 4′,6-Diamidino-2-phenylindole (DAPI) nucleic acid stain (diluted at 1:5000 in distilled water; Thermo Fisher Scientific) was applied to the sections and washed, and a blocking reagent (Protein Block Serum-Free; Dako Japan, Tokyo, Japan) was used to observe the sections. The intermediate portion of the ES was analyzed in IHC. Cultured MDCK cells were used as a positive control for tricellulin and ZO-1 detection. Sections that omitted the primary antibodies were prepared for negative control. Samples from 15 mice were used to confirm the accuracy and reproducibility of the procedure.

**Tracer Permeability Assay**

Animals (5-week-old *Tric−/−* mice) were anesthetized using 2.0% isoflurane, and the cochlear ducts were perfused through the stria vascularis with 10 μl of EZ-Link Sulfo-NHS-LC-Biotin (10 mg/ml, molecular weight: 556.59; Thermo Fisher Scientific) in PBS for 10 min. After 30 min, mice were perfused with a fixative solution of 4% PFA in PBS for approximately 5 min, and the temporal bones were removed. After overnight fixation at 4°C, the temporal bones were decalcified with 0.12 M EDTA
in PBS for 7 days at 4°C. Samples were embedded, and frozen sections (9 μm thick) were made. Streptavidin–Alexa Fluor 488 conjugate (Thermo Fisher Scientific) was used for detection of biotin after refixing and blocking of the sections. The nuclei were counterstained with DAPI.

RESULTS

RT-PCR and Sequencing
PCR products from the WT LCM–ES showed a DNA band of Tric (106 bp) by electrophoresis (Figure 1). RT-PCR from the WT mouse kidney detected Tric expression as a positive control, and no detection from the template replaced with water. Each sequence of PCR products was found to be similar as the initial design.

Immunohistochemistry
The tricellulin expression in the WT mouse ES was then verified by IHC with counterstaining by ZO-1 (Figure 2). ZO-1 was observed uniformly in the epithelial cell junctions of both Tric−/− and WT ES. Staining for tricellulin was observed at the tricellular corners of the epithelial cells in the ES from the WT mouse (Figure 2, arrows). In contrast, no staining for tricellulin was observed from the Tric−/− mouse. Intense staining of tricellulin was confirmed at tTJ in cultured MDCK cells as a positive control.

H&E Staining
The ES of Tric−/− mice was stained with H&E and showed no anatomical and morphological anomalies compared with that of WT mice (Figure 3a and b). From the endolymphatic duct, proximal to the distal part of the ES, the epithelia were maintained in the regular one-layer sac structure. The scala media in the cochlea of Tric−/− mice did not show findings of endolymphatic hydrops, such as bulging of the Reissner’s membrane (Figure 3c).

Tracer Permeability Assay
For examination of the barrier function to macromolecules in the ES of Tric−/− mice, biotin (molecular weight: 556.59) was infused into the endolymphatic space. In the ES of Tric−/− mice, biotin was confined in the endolymphatic space, and no apparent biotin leakage through the epithelia was found (Figure 4). Biotin was also detected in the lumen of the semicircular canal. These findings suggested that the barrier function against biotin in the Tric KO ES epithelium was maintained.

DISCUSSION
Tricellulin is characterized as a predominant protein in the tTJs and is also present in the bTJs, although to a lesser extent. It is considered to exist ubiquitously on a variety of tissues and organs [7,13,14], including the vestibule and cochlea [9], for maintaining paracellular barrier function. As expected, the tricellulin expression in the WT mouse ES was confirmed in both RT-PCR (Figure 1) and IHC (Figure 2). It has been reported that horseradish peroxidase (molecular weight: 40,000) injected in the ES lumen of guinea pigs is impermeable at the paracellular space, suggesting that there is a tight barrier to the...
macromolecules in the epithelial linings of the ES \cite{15}. In the present study, the tracer assay using Tric−/− mice revealed that there was no leakage of biotin (molecular weight: 556.59) from the lumen of the ES (Figure 4). These results suggested that tricellulin in the ES participated in the formation of TJs as one of the barrier function components, but that it is not necessarily crucial for sealing macromolecules in the lumen in the ES. In addition, the morphology of the Tric−/− mouse ES remained normal (Figure 3), with no indication of endolymphatic hydrops. These findings were compatible with the observation that Tric−/− mice show no disturbance in the sense of equilibrium, \cite{10} and the fact that TRIC gene (DFNB49) mutations lead to hearing loss with no obvious vestibular phenotype in humans \cite{9, 16, 17}.

Figure 3. a-c. Hematoxylin and eosin staining in the ES and cochlea of Tric−/− mice. a. ES in Tric−/− mice; b. ES in WT mice; c. cochlea in Tric−/− mice. The ES in Tric−/− mice (a) had no anatomical and morphological anomalies compared with the ES in WT mice (b). The epithelium was maintained in the regular one-layer sac structure. The intermediate portion of the ES was partially surrounded by bone (OP) and showed no apparent ballooning of the sac. The cochlea in Tric−/− mice had no findings of endolymphatic hydrops (c).

ES: endolymphatic sac; Tric−/−: tricellulin-knockout; WT: wild-type; L: lumen of the ES; SC: semicircular canal; SV: scala vestibule; SM: scala media; ST: scala tympani; RM: Reissner's membrane; OP: operculum. Scale bar: 100 μm.

Figure 4. a, b. Tracer permeability assay in Tric−/− mice. Biotin chemical compounds were perfused into the endolymph through the stria vascularis to the cochlear ducts. In the ES of Tric−/− mice, biotin was confined in the endolymph (arrowheads), and there was no leakage through the epithelia. Biotin was also detected in the lumen of the semicircular canal (a). Biotin was detected using a streptavidin–Alexa Fluor 488 conjugate.

Tric−/−: tricellulin-knockout; ES: endolymphatic sac; L: lumen of the ES; SC: semicircular canal. Scale bar: a: 100 μm, b: 20 μm.
**Tric** mice have no functional or structural phenotype not only in the ES but also in the majority of organs, such as the colon, intestine, liver, heart, thyroid, and kidney [10]. Moreover, the paracellular permeability of the stria vasularis in the cochlea is maintained [10]. These data support the previous proposal that tTJs are essential for maintaining the function and structure of a specific part of an organ, such as the organ of Corti in the cochlea, where a strict epithelial barrier system is required [11, 12]. Nevertheless, a question still remains on the phenotypic difference between ES and cochlea, in spite of the fact that they are connected through the same isolated endolymphatic space. One possible explanation is compensation of minor ion leakage by active ion transport. At the organ of Corti in the cochlea of Tric−/− mice, the minor leakage of K+ from endolymph to perilymph is presumed to be the trigger of eventual hair cell degeneration [10]. The cochlear endolymph has a distinctively high concentration of K+ (150 mM) and a positive potential of 80 mV referred to as the endocochlear potential, which is meticulously maintained and indispensable to cause depolarization of hair cells. On the other hand, the ES endolymph contains high Na+ concentration of 103.3 mM and the ES potential of 14.7 mV[10]. Although minor leak of K+ could also exist in the Tric−/− ES, it may be unremarkable since the concentration of K+ in the ES endolymph is smaller (11.6 mM) than that in the cochlea, and K+-electrochemical gradient can be easily reversed even under the resting potential in the ES [19]. Similarly, active Na+ transport by the Na+/K+-ATPase is capable enough to compensate the effects of small Na+ leakage from the ES endolymph in Tric−/− mice [20].

**CONCLUSION**

The tricellulin expression was confirmed in the ES, although the tricellulin deletion had no adverse effects on the morphology and permeability to macromolecules in the ES. The cochlear hair cells are the only disrupted sites resulting from Tric KO as reported previously [10], despite the fact that the ES and scala media in the cochlea share a single endolymphatic space. These results suggest that the effects of solute leakages in the ES of Tric−/− mice may be very limited and compensatable by active ion transport of the ES, or that the ES epithelia may have another sealing system covering the lack of tricellulin in the tTJs [21]. Further studies are needed to elucidate the function of the TJs in the ES.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of the Animal Care and Use Committees of Kagawa University and Kyoto Prefectural University of Medicine.

**Informed Consent:** N/A.

**Peer-review:** Externally peer-reviewed.


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**Conflict of Interest:** The authors have no conflict of interest to declare.

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**European Position Statement on Diagnosis, and Treatment of Meniere’s Disease**

Jacques Magnan, O. Nuri Özgirgin, Franco Trabalzini, Michel Lacour, Antonio Lopez Escamez, Mans Magnusson, Enis Alpin Güneri, Jean Philippe Guyot, Daniele Nuti, Marco Mandalà

Meniere Disease keeps challenges in its diagnosis and treatment since was defined by Prosper Meniere at the beginning of 19th Century. Several classifications and definition were made until now and speculations still exist on its etiology. As the etiology remains speculative the treatment models remain in discussion also.

The European Academy of Otolaryngology and Neurotology Vertigo Guidelines Study Group intended to work on the diagnosis and treatment of Meniere's disease and created the European Positional Statement Document also by resuming the consensus studies on it. The new techniques on diagnosis are emphasized as well as the treatment models for each stage of the disease are clarified by disregarding the dilemmas on its treatment. The conservative, noninvasive and invasive therapeutic models are highlighted.

**KEYWORDS:** Meniere's disease, treatment, betahistine, neurectomy, intratympanic treatment, diuretics, enedolymphatic sac surgery, intratympanic gadolinium, videohead impulse test

**INTRODUCTION**

Meniere’s disease (MD) is a heterogeneous group of disorders defined by three core symptoms: episodic vertigo, tinnitus, and sensorineural hearing loss. The relevance of defining the diagnosis and treatment of MD could not be significantly achieved, as there still appear many arguments and few randomized double-blind prospective studies in this regard. The definition and classification have showed several revisions as the proposal made by the Barany Society in 2015 has received a significant support.

The working group on vertigo guidelines established by the European Academy of Otolaryngology and Neurotology (EAONO) Otologic Guidelines began studying on MD in 2011, and the group members met several times to discuss and offer the EAONO consensus on the diagnosis and treatment of MD. A comprehensive literature search was performed using PubMed and Embase as well to conclude this review.

The evidence has been low in many aspects of diagnosis and treatment options in MD and because of this the EAONO working group needed to make this review for a better clarification.

*Documented by European Academy of Otolaryngology & Neurotology (EAONO) Working Group on Vertigo Guidelines
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Meniere's disease is characterized by episodic vertigo, low frequency fluctuating sensorineural hearing loss, tinnitus, and fullness on the affected side. Gait problems, postural instability, and drop attacks may accompany.

There has been the consensus with the published temporal bone studies that Meniere's disease has signs of endolymphatic hydrops. However, saccular endolymphatic hydrops can be found also in 10% of normal subjects and in 40% patients with >45 dB sensorineural hearing loss without any vestibular symptom [10].

Gurkov et al [3] categorizes endolymphatic hydrops as “primary hydropic ear disease” (PHED) and secondary hydropic ear disease (SHED). The primary disease is still assumed to be idiopathic and covers the whole inner ear. The term “secondary hydropic ear disease” describes the conditions that cause hydrops of the inner ear secondarily (such as endolymphatic sac tumors). This needs to be defined by imaging techniques.

In contrast to the AAO-HNS criteria published in 1972, 1985, and 1995, Barany Society in collaboration with AAO-HNS, the Japan Society for Equilibrium Research, the EAONO, and the Korean Balance Society published the criteria for the diagnosis of Meniere's disease. The Definite Meniere's disease is characterized with episodic vertigo and fluctuating low to medium frequency sensorineural hearing loss, fullness, and tinnitus being manifested at least with two episodes. The duration is mentioned to be between 20 min to 12 hours. Hearing loss in close temporal relationship to the episodes should also be considered [11].

Meniere's disease showed comorbidities with several disorders including autoimmune diseases and migraine [4, 5]. Several lines of evidence support that genetic factors contribute to phenotype variations [6]. Some patients (as high as 10%) may have first and second-degree relatives confirming the familial aggregation [6, 7]. Most of these families show an autosomal dominant pattern of inheritance with incomplete penetrance and variable expressivity [8, 9].

The Ménière's Disease Consortium (a European multicenter initiative to collect clinical data and biological samples) have conducted 2 large epidemiological studies using cluster analyses and it has identified 5 subtypes of MD disease in patients with uni or bilateral involvement [10, 11]. In unilateral MD, group 1 was the clinical variant most frequently observed (53%) and it included patients without a familial history of MD, migraine, or autoimmune comorbidity; MD type 2 was termed delayed MD and was found in 8% of cases and characterized by SNHL which antedated the vertigo episodes; familial MD or type 3 (13%) included all familial cases of MD; MD type 4 (15%) was associated with migraine with or without aura, and MD type 5 (11%) was defined by a concurrent autoimmune disorder [11]. Moreover, the allelic variant rs4947296 is associated with bilateral MD and it has been found in 18% of patients with a comorbid autoimmune disorder [12]. When this advanced diagnosis can be achieved, MD should be treated according to its subtype characterization.

Assessment
Low to medium frequency sensorineural hearing loss as mentioned above is the most significant finding of MD. Therefore, an audiologic evaluation following a relevant history taking is mandatory for diagnosis of MD. Recurring and fluctuant characteristics of the hearing loss pattern is important to mention. The bedside eye movement evaluation represents a fundamental diagnostic step both in the first stage and during the follow-up.

Vestibular test battery
The methods to assess MD have now been enriched with new laboratory techniques. Videonystagmography (VNG) replaced electronystagmography, as it gave the opportunity of realtime observation of nystagmus with its third dimension. Caloric tests are still applicable.

Video head impulse tests are based on analyzing the vestibulo-ocular reflex with two parameters; gain and presence of overt/covert saccades. It is significantly the parameter of peripheral disease and can give access for evaluation of all semicircular canals individually. Video head impulse tests and caloric tests by VNG are the tests not competing but are complementary to each other possibly because they test different frequency parts of the vestibular function [13].

Vestibular evoked myogenic potentials (VEMPs) help evaluate the function of the utricule and saccule as well as the superior and inferior vestibular nerves. VEMPs are the reflexes rising as a response obtained through the sternocleidomastoid and orbital muscles due to high intense acoustic stimuli. These can either be applied as bone conduction or air conduction to stimulate the otolith organs. Today VEMPs are rather used for monitoring the otoolith function and the effect of intratympanic gentamicin applications [14].

Electrocochleography was supposed to be the most specific test to diagnose Meniere's disease for a long time. As it detects the summating and action potentials (SPs and APs) arising from the cochlea and the nerve due to the click stimulations, the belief of elevation of the SP/AP ratio in hydrops populated this evaluation technique. There were difficulties in obtaining the evoked responses, as the ideal location was promontorium, which was not practical to put electrodes nearby the round window routinely in office-based conditions, and the response quality has been low with the tympanic membrane surface electrodes. Electrocochleography has lost its popularity over time [15].

Imaging
In 2007, Nakashima et al [16] proposed 3 Tesla magnetic resonance imaging (MRI) evaluation of the inner ear following intratympanic gadolinium injection. Gadolinium that perfuses through the round window membrane allows the boundary between the endolymphatic space and the perilymphatic space to be distinguished.

MRI with intravenous (IV) administration of gadolinium has also been suggested. A delay of 4 hours is necessary following the injection of double dose of gadolinium. Both ears can be assessed but there is the risk of systemic toxicity due to the high dose of gadolinium [17].

While the T2-weighted images represent both perilymphatic and endolymphatic fluids, the bright signal on the 3D-FLAIR images represents only the perilymphatic fluid and internal dark signal represents the endolymphatic fluid [18].
In case the endolymphatic duct expands more than 33%, it should be argued as endolymphatic hydrops. However, the visualization of endolymphatic hydrops is not required to define MD and the MRI imaging should not be used to replace the diagnostic criteria of MD when also all definition criteria are fulfilled.

Firstline Management (Preventive)
A personalized approach for MD patients is strongly recommended. So, if a patient presents a comorbid condition such as allergy, migraine or autoimmune arthritis, they should be treated. The familial history of hearing loss and episodes of vertigo are also recommended, since genetic testing will identify the causal variant in 30% of familial cases, paving the way for gene therapy in few years.

Diet
The known adverse effects of caffeine and salt in MD is not clear. Low sodium diet and high water intake may prevent the release of vasopressin and help to maintain inner ear homeostasis [19,20]. The AAO-HNS scale restricts caffeine in MD with the argument that caffeine can provoke modifications in the endolymph volume with its sympathomimetic action. The habitual consumption of caffeine varies due to the geography; hence, the relation of habitual intake of caffeine and Meniere's disease symptoms also differ. It is possible to assume that low amounts of caffeine, such as 100 mg/day, will not trigger Meniere's symptoms [21].

Betahistine
Betahistine is a weak histamine H1 agonist and a stronger H3 antagonist. This is the medication currently used worldwide except for the USA. There have been remarkable studies about the efficacy of betahistine on reducing the vertigo episodes of MD, and some studies suggest its dosedependent effect in suppressing the frequency of vertigo attacks [22-25].

Furthermore, there are others, such as the Cochrane reviews, which support the positive effect of the medication on reducing the symptoms with good tolerance, also by arguing significant methodological limitations over the conducted studies; hence, larger studies for reaching to higher quality evidence on suggesting the use of betahistine [26].

A meta-analysis by Nauta [27] suggested the therapeutic benefit of betahistine in Meniere's disease.

The recently conducted multicenter study also known as BEMED suggested that two different doses (48 and 144 mg/day) of betahistine did not show any difference from placebo regarding the incidence of attacks and vestibular function [28].

The conflicting findings among different studies motivate further studies with well-defined inclusion and exclusion criteria and higher doses of betahistine to be accomplished. According to the clinical experience, the use of Betahistine 48 mg bid for 3-6 months to prevent Meniere's attacks can be advised.

Diuretics
The Cochrane report by Burgess & Kundu (2006) identified ten trials executed on diuretics' effect and among them two were placebo-controlled. As all were lacking the high quality of evidence, some studies have reported the efficacy of diuretics. The report concluded that there has been no good evidence of using diuretics in MD [29].

Diuretics are generally issued as first-line therapy for MD. The studies that support using diuretics have a low level of evidence [30]. The thiazide group diuretics can be a part of the medical treatment.

Secondline Management (Preventive)
In case medical treatments and refraining from excess of caffeine and salt does not control Meniere's episodes, a second-line treatment must be considered.

Intratympanic treatment has been very popular since the last two decades as being practical to apply even in the office setup.

Among the two available steroids derivatives, dexamethasone is practical to use due to better tolerance by the patients, as methylprednisolone creates burning sensation in the middle ear mucosa. The challenge with dexamethasone is its availability with low concentrations, such as 4 mg/mL.

The studies executed on application of intratympanic steroids for MD show any homogeneity regarding the treatment protocols. Lavigne et al. [31] could only find one article being in favor of controlling tinnitus and vertigo in Meniere's disease. Being safe in terms of complications, such as hearing loss, has been the main advantage of using steroids. Individual based application of intratympanic dexamethasone can be favored.

Beyea et al. [32] reported that the effect of intratympanic dexamethasone application can have a shortterm control over the Meniere episodes as being effective in only 5% to avoid ablative surgery.

The Cochrane review by Westerberg [33] showed limited evidence to support the effectiveness of intratympanic steroids in MD treatment. Of note, the recent Oto-104 study with 12 mg dexamethasone can have the potential of discarding the disadvantages of intratympanic dexamethasone treatment regarding its low concentration [34].

Thirdline Management
Endolymphatic sac surgery was first defined by Portmann in 1927. There have been several discussions in favor and against this technique. The most remarkable argument against endolymphatic sac surgery was introduced by Jens Thomsen [35], which mentioned that the procedure has only a placebo effect.

The evidence level to support this surgery is low. Additionally, there are welldesigned randomized, double-blind, placebo-controlled studies for it [36].

The Cochrane review by Pullens et al. [37] over two randomized controlled studies showed that no significant effect could be achieved using the endolymphatic sac surgery, providing insufficient evidence for the beneficial effect.

Kitahara [38] proposed the injection of dexamethasone into the sac. As the endolymphatic sac is the only location for immune reactions in the temporal bone the hypothesis by Kitahara makes sense. In a retrospective study, Wick et al. suggested that endolymphatic sac shunt procedures may benefit from steroid instillation at the time of shunt placement [39].
Intratympanic Gentamicin Injection

Gentamicin is an aminoglycoside antibiotic having more vestibulotoxic than cochleotoxic effect. Its effect is mainly causing atrophy on type 1 vestibular cells as well as the neuroepithelium [40].

Although the intratympanic application of gentamicin poses the risk of hearing loss, many clinical studies have been designed to find out the lowest risk of its application with the maximum control of vertigo in MD. Hence, due to the toxic effect over the peripheral vestibular end-organ, dizziness and unsteadiness following the injection can be a minor problem that can be resolved by vestibular rehabilitation [41].

The intratympanic application of gentamicin has received more interest due to its strong effect over the Meniere episodes, that also beat the frequency of vestibular neurectomies.

The recommended application of gentamicin is one injection of 26.7 mg/mL concentration and scanning the vestibular physiological responses by the number of vertigo spells, a bedside evaluation, VEMP, and video head impulse tests.

Fifthline Management

Advanced Surgery

Among the treatment techniques the only methods for MD that have gained high evidence are labyrinthectomy and vestibular neurectomy. Among these two, vestibular neurectomy is a selective technique issued to superior and inferior vestibular nerves and keeping the cochlear nerve safe. The efficiency of both techniques is good [42].

Vestibular neurectomy is believed to be the most efficient technique for drop attacks (Tumarkin’s disorder) and for incapacitating Ménière’s disease.

Labyrinthectomy is the oldest surgical method to treat MD, and today is limited to older patients. The technique can be associated with cochlear implantation within the same stage in case of profound bilateral hearing loss [43].

CONCLUSION

The definition of MD has reached a large international consensus, diagnosis and especially treatment still represent a debated topic. The main aim of this position paper is to identify a common path for medical professionals dealing with Meniere's disease diagnosis and treatment based on literature evidences and expert opinions.

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REFERENCES


Contemporary Molecular Biology of Sporadic Vestibular Schwannomas: A Systematic Review and Clinical Implications

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In light of missing systematic reviews in the literature, the objective of this paper is to present the contemporary knowledge on the molecular biology of vestibular schwannomas (VS), based on a systematic literature search. In addition, current and prospected medical therapy based on molecular biology is addressed. A systematic literature search was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. The systematic search was performed in the Pubmed and Embase databases. The following were the words searched: acoustic neuroma/vestibular schwannoma, molecular biology, gene, and microRNA. Specific inclusion and exclusion criteria were determined prior to search. The systematic search rendered 486 articles, ultimately yielding 69 included articles, whereas 35 were from relevant references. The occurrence of at least one mutation in the merlin gene was reported to range between 54% and 76%, whereas the loss of heterozygosity (LOH) corresponding to chromosome 22 occurs in 25% to 83% of sporadic VS. Global gene expression studies indicate that a number of genes other than merlin are at play. No high-level methylation of the merlin gene has been found. Several miRNAs are deregulated in tumor tissue, among others let-7d, miR-221, and miR-21. The acquired knowledge on molecular biology has led to several clinical implementations. Lack of the tumor suppressor merlin plays a principal role in the development of VS. Existing knowledge on the molecular biology has led to the first attempts of targeted medical treatment to prevent tumor growth. Future research is likely to introduce potential imaging markers with prognostic value and new targets for medical therapy.

KEYWORDS: Acoustic neuroma, molecular biology, gene expression, microRNAs, review

INTRODUCTION

Vestibular schwannomas (VS) arise from the Schwann cells, sheathing the vestibular branch of the eighth (VIII) cranial nerve. Although these tumors are histologically benign, they may cause hearing loss, tinnitus, and facial palsy, and if growing rapidly to a large size, even brainstem compression and death. VS can occur unilaterally or when associated with neurofibromatosis type 2 (NF2), bilaterally. The mutual molecular hallmark of both NF2-associated VS and sporadic VS is biallelic inactivation of the merlin gene, also known as the NF2-gene, which is a tumor suppressor located at 22q12 (1). Several genetic and epigenetic aberrations have been shown in sporadic VS, for example, various mutations in the merlin gene, loss of heterozygosity (LOH) on several chromosomes, deregulation of other genes, abnormal microRNA expression pattern, and CpG island methylation. This review summarizes the contemporary molecular biology of sporadic VS via systematic literature searches, as well as addressing current and prospected medical therapy based on molecular biology.

METHOD

Overview of Systematic Literature Review
A systematic literature review was conducted to identify published studies regarding merlin gene mutations, deregulated gene expression, LOH, DNA methylation, and deregulated microRNAs in sporadic VS.
Systematic Search Strategy and Terms
The objective of our search strategy was to identify relevant studies concerning the molecular biology of sporadic VS. First, Pubmed, Embase, and the Cochrane Center were searched for previously published reviews based on a systematic search of the literature. None were found. Second, the same sources were searched systematically for publications on the molecular biology of sporadic/unilateral VS, using both simple search strings as well as Mesh terms. No limitations besides the inclusion and exclusion criteria were employed. The search was conducted on October 1, 2017.

The search was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [2].

Pubmed
- "Neuroma, Acoustic" AND "Genes"
  - 109 hits—36 articles of interest
- MESH ("Neuroma, Acoustic"[Mesh]) AND "Genes"[Mesh]
  - 311 hits—48 articles of interest
- MESH ("Neuroma, Acoustic"[Mesh]) AND "MicroRNAs"
  - 6 hits—5 articles of interest
- MESH ("Neuroma, Acoustic"[Mesh]) AND "Molecular Biology"[Mesh]
  - 13 hits—1 articles of interest

Embase
- Acoustic Neurinoma/ AND Gene/
  - 35 hits—4 articles of interest
- Acoustic Neurinoma/ AND microRNA/
  - 5 hits—2 articles of interest
- Acoustic Neurinoma/ AND Molecular Biology/
  - 7 hits—0 articles of interest

Inclusion and Exclusion Criteria
Inclusion criteria were as follows: (1) analysis performed on unilateral/sporadic VS; (2) analysis on human tissue; (3) published in the English language.

Exclusion criteria were as follows: (1) not article; (2) not original research; (3) case reports.

Implementation of the Search Strategy and Study Selection Process
An initial review of the search results was conducted. All the articles were initially screened for relevance based on their titles and abstracts. Any report not excluded by this process was included for full-text analysis. The reports were then subjected to full-text analysis, using the above inclusion and exclusion criteria.

Data Extraction Process
Data were extracted by thorough examination of the included articles, on information regarding the methods used, tissue analyzed, and results. Articles containing both sporadic VS and NF2 associated VS were evaluated. If the results were divided and a clear distinction between the two groups was possible, the article was included.

Five categories were created to describe the molecular biology of sporadic VS: the merlin gene mutations; LOH; deregulated genes; DNA methylation; and microRNA.

Risk of Bias
The search and data extraction were intended to be as unbiased as possible. No attention was given to the author, source, country, or other distinctive criterion in our initial searches. The inclusion and exclusion criteria were all defined prior to the search. We received no outside funding and had no competing interests regarding the review.

Quality Assessment
All full-text articles reviewed were also informally evaluated on the quality of materials, methods, and results. No studies were excluded on the grounds of poor-quality methods or due to non-substantiated results.

RESULTS and DISCUSSION
After a systematic literature search and inclusion/exclusion of papers based on the above criteria, 33 articles were included in the review. From these 33 articles, another 35 articles were included from relevant references. A PRISMA diagram displaying the search is shown in Figure 1. Based on these 68 articles, a synopsis on the contemporary knowledge on the various aspects of the molecular biology of VS is given below. In addition, current and prospected medical therapy based on molecular biology is addressed. A meta-analysis was not possible due to the heterogeneity of methodology in the study reports.

The Merlin Gene
The protein merlin is a tumor suppressor encoded by a gene located at the chromosome 22q12.2. Silencing of the merlin gene is the
possible loss of expression of p21 in sVS has been shown \[10\]. In addition, merlin exerts inhibitory effects on multiple mitogenic signaling pathways. The loss of functional merlin on the other hand also activates many signaling pathways. The contribution of these pathways to tumor pathogenesis and maintenance is, however, not fully understood \[8, 11\].

Merlin Gene Mutations
In NF2-associated VS, one of the germline merlin alleles is inactivated, and the development of NF2-associated VS therefore only calls for an additional mutation, allelic loss, or silencing of the other allele \[12\]. In sVS, however, somatic bi-allelic merlin gene inactivation seems to be necessary for the formation of the tumor \[13\].

Mutations of the merlin gene consist of insertions, deletions, and single-base substitutions resulting in frameshifts and nonsense mutations \[14, 15\]. The occurrence of at least one mutation in the merlin gene coding sequence has been reported to range between 54% and 76% \[13-23\]. The consequence of a mutation is mainly the synthesis of truncated proteins (75%-93%) \[14, 18\]. In the studies by Torres-Martin et al. \[16\] and Bian et al. \[17\], the majority of the mutations occurred in the first half of the 17 exons in the merlin gene. Welling et al. \[18\] found another mutational distribution, as all mutations occurred in the exon 4–6 in the NF2-associated tumors, and nine of ten mutations occurred in exon 7–11 in sVS, which is supported by the findings of Jacoby et al. \[22\]. Chen et al. \[24\] found a higher percentage of the NF2 mutations in young patients (age under 20) with 66.7% of 12 patients compared to 34.5% of 145 adult patients. All mutations in young patients were truncated mutations. This may indicate that NF2 mutations may play a role in the formation of early sVS formation; however, the data sample was rather small, and the conclusion is that further research is needed.

Reported differences may be due to the selection bias and variable methodology. However, as mutation(s) are not occurring in almost all cases, other factors need to be at play for the development and maintenance of a tumor.

Loss of Heterozygosity
LOH is the loss of function of one allele, after the other allele has already been inactivated. Several mechanisms may account for LOH, for example, deletion resulting in a loss of a chromosome segment, mitotic recombination, translocation, and gene conversion.

Early studies showed that only 17% to 22% of sVS had a LOH on chromosome 22, whereas later studies using more sensitive methods report LOH in 25% to 83% \[13, 15, 17, 23, 25-30\]. The occurring differences may again be due to selected patient materials or differences in unbalanced chromosomal abnormalities versus balanced chromosomal abnormalities. Regardless of variable occurrence, the studies do however confirm that LOH is a frequent feature of sVS, and it is therefore likely to be involved in both tumor development and maintenance.

LOH at other chromosomes may also play a role in tumor pathogenesis. In six of 14 sVS (43%), Dayalan et al. \[36\] found LOH on chromosome 17p, which among other genes codes for tumor protein 53 (TP53). However, an overexpression of TP53 mRNA and TP53...
protein was seen in all tumors. This phenomenon has been demonstrated in earlier studies of other neoplasms, for example, breast cancer.\(^{37}\) However, Monoh et al.\(^{38}\) found neither mutations nor LOH on chromosome 17p. They concluded that TP53 does not play an important role in the tumorogenesis or maintenance, thus contradicting Dayalan et al.\(^{36}\).

**Deregulation of Other Genes**

Four larger studies have explored global gene deregulation in VS, whereas other studies have focused on specific, selected genes.\(^{16, 19, 39-50}\) Different techniques have been used and only a few common findings exist between these studies.

Welling et al.\(^{39}\) pioneered, Caye-Thomasen et al.\(^{40}\) followed, and later Aarhus et al.\(^{44}\) and Torres et al.\(^{17}\) joined in analyzing the global gene expression in VS. They all used cDNA microarrays, although platforms, number of tumor samples, as well as number and type of control tissue differed. Welling et al. found the same 42 genes up-regulated in five VS, and eight genes significantly down-regulated in all the seven analyzed VS, compared to control nerve tissue. Five of the up-regulated and one of the down-regulated genes recur in later publications. The findings share similarities with the expression data published by Cayé-Thomasen et al., who included 16 sVS and compared them to three normal vestibular nerves. Seventy-five genes were up-regulated, and three were down-regulated. Eight of the up-regulated genes recur in other publications, whereas none of the down-regulated genes have been found by other authors. The gene Osteonectin/SPARC was found to be up-regulated by Welling et al.\(^{39}\) and Aarhus et al.\(^{19}\), but it was not deregulated in the study of Cayé-Thomasen et al. However, the scavenger receptor stabilin 1, which mediates targets for degradation by Osteonectin/SPARC, was up-regulated.

Aarhus et al.\(^{19}\) conducted the first of the bigger studies to validate their findings with qRT-PCR. Five of the up-regulated and four of the down-regulated genes had also been identified in the other studies. Ingenuity pathway analysis was used to show that ERK was the primary network for the deregulated genes. They also found that the analyzed VS were divided into two groups regarding the mRNA expression. These findings were replicated by Torres-Martin et al.\(^{14}\) who analyzed 28 sVS and compared them to nine various types of control nerve tissue. Torres-Martin et al. also found that the tyrosine-protein kinase Met (cMET) pathway, possibly enhanced by an upstream signaling of SPP1 and CAV1 among others, appears to play a principal role in the formation and preservation of VS. A possible hormonal influence was also discovered, as the androgen receptor was deregulated.

The cMET pathways and the possible cross-talk with VEGF-A were investigated by Dilawi et al.\(^{53}\), who found that both cMET and VEGF-A are significantly overexpressed in sVS compared to non-neoplastic Schwann cells. A knock-down of either VEGF-A or cMET reduced the other, and inhibition of the cMET pathway reduced the proliferation in sVS cells. The cMET pathway must therefore be considered a potential target for therapeutic therapy.

Sass et al.\(^{32}\) pioneered by doing a global gene expression analysis in fast-growing sVS compared to slow-growing sVS using a DNA microarray. Several notable genes were up-regulated in the fast-growing tumors, for example, the erbb2 interacting protein (Erbin), platelet-derived growth factor C, phosphatidylinositol 3-kinase, actinin alpha 1, and several toll-like receptors. Notable down-regulated genes included the brain specific protein and neuronal cell adhesion molecule 1. The ingenuity pathway analysis (IPA) was performed, and a number of canonical pathways were found to be related to viral infections. This supports the notion that there could be a viral etiology behind the pathogenesis and especially tumor growth of sVS. Functional molecular networks derived from the IPA demonstrated the importance of PI3K for the growth as it was in four of the five top functional molecular networks. The top network, including 23 of the total 109 deregulated genes in the study, found NF-κB and P38 MAPK to be the hubs of the network, and thus involved in several interactions with the deregulated genes. NF-κB inhibits apoptosis and is up-regulated in multiple cancers, whereas P38 MAPK may contribute to VS development and progression through an effect on Schwann cell differentiation. Dilawi et al.\(^{53}\) previously completed the first functional molecular network analysis of sVS in 2015 and found that the same NF-κB was the center hub of their functional molecular network. This indicates that NF-κB is involved not only in the formation, but also the growth rate of sVS. Dilawi et al. also found that cyclin D1, Bcl2 and gene TNF encoding TNF, was overexpressed in sVS compared to control nerve in the same study.

In minor studies regarding the gene expression, the AKT1 gene, Erbb2, Neuregulin 1, and the EGF-receptor were found up-regulated and the AKT pathway to be active.\(^{44, 45, 54}\) The immunoreactivity for EGFR was also found, however, deemed as not important due to low expression levels as well as a small tumor sample.\(^{46, 47}\) Cayé-Thomasen et al.\(^{46}\) found a positive correlation between VEGF, VEGFR-1, and tumor growth rate, while Moller et al.\(^{55}\) found a positive correlation between MMP-9 and the tumor growth rate. Seol et al.\(^{43}\) found a down-regulation of p27 in 67% of aggressive VS compared to 20% in non-aggressive tumors. O’Reilly et al.\(^{41}\) found an overexpression of FGFR1 in growing sVS. Hence, VEGF, VEGFR-1, p27, and FGFR1 may play a role in the growth of sVS, and may be potential prognostic markers or therapeutic targets.

The Cyclin D1 expression, HIF-1α/α, Epo, EpoR, and bcl2 expression have also been found in tumor samples, as well as the silencing/hypermethylation of RASSF1A, which is associated with negative cyclin D1 expression. Hung et al.\(^{42}\) found an up-regulation of the L1 cell adhesion molecule, also found in the latter three of the four larger gene expression studies.\(^{16, 19, 40}\) Chen et al.\(^{14}\) investigated the difference of Cyclin D1, merlin, phosphorylated merlin, and p53 expression between young patients under 18 and adults, with no significant difference.

De Vries et al.\(^{49}\) analyzed 48 sVS for the 13 most frequent mutations in BRAF, EGFR, PIK3CA, and KRAS. No mutations were found, suggesting that these genes do not play a role in tumor development.

Sirén et al.\(^{42}\) found an overexpression of urokinase plasminogen activator and tissue plasminogen activator (tPA) in 13 sVS samples.
They also found up-regulation of the PA-PAI-I complex leading to reduced tPA activity compared to NF2-associated VS. This indicates that sVS may be more prone to hemorrhaging and thrombosis and are less likely to be invasive.

Overall, the studies on deregulated genes in sVS are relatively few, and the different methods/arrays used make comparisons difficult and overall conclusions uncertain. The MET-pathway as well as NF-kB could represent potential targets, as they appear to be principal in the development and preservation of sVS.

DNA Methylation
In sVS, the functional merlin is not present, although as many as 40% of the tumors contain an intact wild-type merlin gene. As a number of human cancers have been associated with abnormal methylation of CpG-islands located in the gene promoter regions, several studies have explored aberrant methylation of the merlin gene promoter regions in schwannomas, as a potential explanation for the lack of functional merlin.

Kino et al. [57] showed that a 70-base pair region of the merlin gene contained five CpG-sites playing a role in the transcriptional silencing of the gene. Fourteen of 23 sporadic schwannomas showed CpG-methylation, with an absence of a transcriptional product in eight of the 14 tumors. Gonzalez-Gomez et al. [58] found merlin gene methylation in 19% of 27 sVS. In these studies, there were no correlations with merlin gene mutations or deletions. Chen et al. [24] investigated the phosphorylation of merlin in young patients compared to adult patients and found that patients with phosphorylated merlin (3/12 patients), exhibited larger tumor size than patients with deficient merlin (9/12 patients). Kullar et al. [59] used a more sensitive pyro-sequencing technique and found no high-level methylation in any of 40 sequenced sVS. Again, a possible explanation for conflicting results may be different patient cohorts and differences in sensitivity between the methods used. Kullar et al. used the most sensitive method and did the most comprehensive study including the copy number and mutational status of each tumor. Consequently, hypermethylation of the CpG-islands of the merlin gene is likely to play little or no role in the silencing of the merlin gene.

Methylation of other genes has also been explored. Both Gonzalez-Gomez et al. [58] and Lassaletta et al. [60] examined the methylation status of a number of genes important for tumor development, including the angiogenesis inhibitor THBS1, the DNA repair protein MGMT, the extracellular matrix binding protein TIMP-3, the caspase and thus apoptosis initiator CASP8, and the apoptosis inducer and cell growth inhibitor TP73. Lassaletta et al. [61] also examined methylation of the gene RASSF1A, a tumor suppressor candidate acting downstream ras, and found that the methylation status was inversely related to tumor growth. However, methylation status of this particular gene may be related to age, which was also found in these study [62, 63].

In conclusion, methylation of the merlin gene is likely to play little or no role in the development of VS, whereas determination of a potential role of methylation of other genes warrants additional studies.

MicroRNA
Two studies have been done on miRNAs in sVS [64, 65]. Cioffi et al. [64] used qRT-PCR and RT-PCR to analyze miR-21 overexpression in eight sporadic VS compared to nine normal vestibular nerves, and five normal greater auricular nerves. They found consistent overexpression of miR-21 and a correlating decrease in PTEN, a known target of miR-21. They also found that anti-miR-21 was related to decreased tumor growth.

Torres-Martín et al. [65] found miR-7 to be up-regulated in their study and has been shown to be deregulated in several other tumors [66, 67]. Torres-Martín et al. [66] analyzed 15 sporadic VS and one NF2-associated schwannoma, comparing with three controls. They found 174 microRNAs to be deregulated, including confirmation of the up-regulation of miR-221, miR-21, miR-29, miR-30a, and miR-138 and the down-regulation of miR-7, miR-638, miR-143, and miR-498. However, let-7d, miR-451, and miR-34a were not deregulated to the extent found in the studies above. They also found up-regulation of hsa-miR-363, which inhibits merlin synthesis post-transcriptional, and a global up-regulation of a cluster of miRNAs in the chromosomal region of 14q32 that might contribute to the development and maintenance of VS. In addition, a difference was found between the miRNA expression pattern in tumors with specific molecular characteristics, such as mutations in the merlin gene and/or the LOH on chromosome 22q, as compared to tumors without specific molecular characteristics.

Clinical Implications
Current treatment options are microsurgical excision, radiotherapy, and watchful waiting. However, due to the growing knowledge on the molecular biology of VS, an increasing number of potential targets for medical therapy are being identified. The PDGF and VEGF are known angiogenetic factors, and several studies have correlated tumor VEGF to either tumor growth or volume, which subsequently spawned the first medical treatment of VS, namely the anti-VEGF antibody Bevacizumab [48-48]. The most recent research in this field have demonstrated that vascular biomarkers from dynamic contrast-enhanced MRI can predict the tumor response to treatment with the VEGF antibody [71]. The PDGF was first considered to be a target for therapy by Altuna et al., and later Ammoun et al. corroborated the potential by the use of a potent PDGF receptor inhibitor [72, 73].

Other therapies have targeted the EGFR/ErbB2, PI3K/AKT, HER-1/EGFR, PAK, and mTORC1, and different degrees of VS shrinkage and hearing improvement have been demonstrated, with some of them still in clinical trials [74-79]. These treatments are tested on both sporadic, as well as NF2-associated VS, with positive results. Dilawri et al. [53] used the NF-kB inhibitor Curcumin on cultures of sVS cells, resulting in a dose-dependent decrease in proliferation.

CONCLUSION
This is the first systematic review regarding the molecular biology of sVS, and the first to include studies regarding miRNA. A lack of the tumor suppressor merlin plays a principal role in the development of VS. As the merlin gene mutations are not found in all tumors, deregulation of other genes and, post-transcriptional silencing by miRNA and/or LOH, are likely factors to play a role in tumor patho-
genesis and growth, whereas a role of DNA methylation appears to be unlikely. Existing knowledge on the molecular biology has led to the first attempts of targeted medical treatment to prevent tumor growth. Additional attempts are imminent, and future expansion of our knowledge on the molecular biology of these enigmatic tumors is likely to introduce potential imaging markers with prognostic value and new potential targets for medical therapy.

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Case Report

Traumatic Facial and Vestibulocochlear Nerve Injury in The Internal Acoustic Canal in The Absence of A Temporal Bone Fracture

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We present a rare case of traumatic facial and vestibulocochlear nerve injury in the internal acoustic canal in the absence of a temporal bone fracture. A 2.5-year-old female presented with sudden-onset left-sided facial paralysis and ipsilateral total hearing loss after being hit by a falling television. High-resolution computed tomography revealed an occipital fracture line that spared the temporal bone and otic capsule. Diagnostic auditory brainstem response testing showed that wave V at 90-dB normal hearing level was absent in the left ear. Needle electromyography revealed severe axonal injury. Facial paralysis regressed to House–Brackmann grade IV 9 months after the trauma, and no surgical intervention was scheduled. Traumatic facial and vestibulocochlear nerve injury can occur in the absence of a temporal bone fracture. Thus, careful evaluation of the internal acoustic canal is mandatory if concurrent 7th and 8th cranial nerve paralyses exist with no visible fracture line.

KEYWORDS: Facial nerve, vestibulocochlear nerve, avulsion, facial paralysis, facial nerve injuries

INTRODUCTION

Traumatic injuries constitute the second most common cause of facial nerve palsy worldwide [1]. Traumatic facial nerve palsy usually occurs after a temporal bone fracture, which is most often caused by motor vehicle accidents and falls [2]. In its intratemporal course, the facial nerve enters the fallopian canal, which is situated between the lateral end of the internal acoustic canal and the stylo-mastoid foramen. Fracture lines involving this canal can cause partial or complete impairment of facial nerve function. Temporal bone fractures are classically described according to the long axis of the petrous bone being classified as longitudinal, transverse, or mixed [3]. Immediate-onset facial paralysis and total sensorineural hearing loss are hallmarks of a transverse fracture involving the otic capsule that transects the facial nerve. Moreover, there is a new classification system based on whether or not the fracture line violates or spares the otic capsule, and it is thought to be a better clinical predictor than the classical classification [4].

Here, we present the case of a patient with immediate-onset facial paralysis and complete deafness caused due to being hit by a falling television. The patient was diagnosed with complete transection of the facial and vestibulocochlear nerve complex in the internal acoustic canal in the absence of a temporal bone fracture. To the best of our knowledge, this is the second such case to be reported in the English-language literature.

CASE PRESENTATION

A 2.5-year-old female presented to our clinic with immediate-onset complete left-sided facial paralysis and total hearing loss due to head trauma caused by a falling television. The accident occurred 1 month prior to the presentation. The patient was referred from another institute for detailed evaluation and surgery, if necessary. Otolaryngologic examination showed House–Brackmann grade VI facial paralysis on the left side. The external auditory canal and tympanic membrane were normal on both sides. Facial nerve
tests were not performed at the referring institute. The parents of the patient provided written informed consent.

High-resolution computed tomography (HRCT) showed a non-depressed occipital cranial fracture line that spared the otic capsule and temporal bone (Figure 1). The facial canal was completely intact up to the stylomastoid foramen, and no injury to the facial nerve was detected along its course. Automated auditory brainstem response (ABR) was absent on the left side, and a diagnostic ABR was performed under general anesthesia. Wave V at 90-db normal hearing level was absent and cochlear microphonic was detected in the left ear (Figure 2). The ABR findings and co-occurrence of the 7th and 8th cranial nerve palsies raised suspicions of a retrocochlear pathology. Thus, a 1.5-Tesla magnetic resonance imaging (MRI) was performed, and constructive interference in steady-state sequences using the T2-weighted three-dimensional gradient-echo technique showed a signal intensity difference between the left side and the unaffected right side, as well as adhesion around the facial and vestibulocochlear nerve complex at the fundus of the internal acoustic canal. The reticular contrasting pattern was interpreted as granulation tissue and/or fibrosis caused by traumatic nerve injury considering the clinical findings and history of the patient (Figure 3).

Concentric needle electromyography (EMG) of the left orbicularis oris and orbicularis oculi muscles was performed at rest and during voluntary contraction 2 months post-injury. Resting EMG showed denervation potentials (spontaneous fibrillation and positive sharp wave potentials) (Figure 4a). There were no motor unit potentials or reinnervation potentials during voluntary activity. The patient’s inju-
ry was graded as severe axonal injury based on these neurophysiological findings. She was scheduled for monthly follow-up to monitor for any signs of spontaneous recovery. Minimal functional recovery of the orbicularis oculi and orbicularis oris muscles was observed 5 months post-injury. The patient’s facial paralysis had regressed to House–Brackmann grade IV at 9 months post-injury. Denervation potentials were determined at rest, as in her previous EMG examination, on her follow-up EMG. Interestingly, during voluntary contraction, polyphasic reinnervation motor unit potentials appeared in her needle EMG reflecting her clinical recovery (Figure 4b). Surgical intervention was not scheduled for the patient because initiation of the spontaneous healing process was observed.

DISCUSSION

Immediate-onset facial paralysis following blunt trauma is most likely associated with nerve transection. Several mechanisms have been proposed to explain the pathophysiology: 1. Temporal fractures involving the fallopian canal and disrupting the facial nerve; 2. Transection of the facial nerve by an adjacent bone spicule; 3. The medially directed vectoral force of the trauma applied to the cranium and brain, leading to avulsion of the nerve [5]. HRCT is adequate for visualizing a temporal fracture line in most cases; however, it may not detect osseous microfractures or bone spicules. Cone beam CT, which has a higher resolution (range: 0.09–0.4-mm slice thickness), might aid in the visualization of microfractures and bone spicules, although it is not available at all institutes. MRI should be used for visualizing nerve integrity, neural ischemia, edema, intraneural hematoma, and possible adjacent dural enhancement. Dural enhancement along the anterior border of the petrous bone, or in the internal acoustic canal, may be an indicator of osseous micro- or macrofractures that cannot be radiologically detected [6].

In the presented case, adjacent dural enhancement was not detected in the internal acoustic canal and the possibility of an unidentified osseous microfracture was excluded. Another possible mechanism for immediate-onset facial paralysis following blunt trauma is transection of the facial nerve by a bone spicule. However, it is unlikely that a bone spicule broke off from its bed in the absence of a temporal fracture in the presented case; moreover, even if we make such an assumption, it would not have been possible for the nerve fibers to reconnect with their conjugate fibers and initiate the regeneration process, as a physical bony obstruction would block the fibers. In the presented case, there were no radiological findings of a temporal fracture line or bone spicule and an initiation of the partial recovery of the facial nerve function was observed.

In the first published case of transection of the facial and vestibulocochlear nerve complex in the internal acoustic canal, it was reported that the facial and vestibulocochlear nerves were avulsed in the internal acoustic canal [5]. Similar to our patient, the patient in the previous pediatric case had experienced blunt trauma during a motor vehicle accident directed toward the ipsilateral occipital region, causing immediate-onset facial and vestibulocochlear nerve paralyses due to injury to the nerve complex in the lateral internal acoustic canal. In the previous case, it was radiologically and surgically observed that both nerves were totally avulsed at the osseous entry point in the fundus of the internal acoustic canal and their proximal ends were medially displaced after the medially directed traction along the nerve bundle. The researchers suggested that because the pediatric cranium is not as rigidly fixed as the adult cranium, due to immaturity of the synchondroses, external forces can mobilize intracranial structures such as the cerebellum and hindbrain medially while displacing the temporal bone outwardly. Thus, the vestibulocochlear and facial nerve complex ruptures, and the proximal fibers get pulled medially. This theory could explain the mechanism of injury in the present case; however, the proximal nerve bundles were not displaced medially, and no significant gap was found between the bundles. This might have happened because the energy associated with a falling television was lower than that associated with a motor vehicle accident in the previous case, and the nerve bundles in the present case fused, initiating the recovery process.

In conclusion, traumatic facial and vestibulocochlear nerve injuries can occur in the absence of a temporal bone fracture. Concurrent
palsy of these two nerves in the absence of a temporal bone fracture may indicate an avulsion injury; therefore, the internal acoustic canal must be carefully evaluated in such cases.

Informed Consent: Written informed consent was obtained from the parents of the patient who participated in this study.

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Case Report

Cochlear–Internal Canal and Cochlear–Facial Dehiscence: A Novel Entity

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Different types of otic capsule dehiscence restricted to the cochlea have been described. Here we describe the case of a patient with a cochlear–internal auditory canal dehiscence associated with a cochlear–facial dehiscence not reported before. A 53-year-old patient with severe to profound sensorineural hearing loss due to bilateral Meniere’s disease underwent a cochlear implant surgery on the right ear. Preoperative brain magnetic resonance imaging findings were reported to be normal; during surgery, a cerebrospinal fluid gusher occurred at the time of round window opening. Postoperative computed tomography imaging showed a bony dehiscence at two levels of the otic capsule.

KEYWORDS: Dehiscence, otic capsule, CSF gusher, cochlear implantation, third window lesions

INTRODUCTION
Since the first report of otic capsule dehiscence in patients with superior semicircular canal dehiscence (SSCD) by Minor in 1998 [1], different types of otic capsule dehiscence restricted to the cochlea have been described: cochlear–carotid, cochlear–internal auditory canal, or cochlear–facial dehiscence [2]. The awareness of this phenomenon is important considering that a dehiscence in the otic capsule opens up a third window. The variety of signs and symptoms that may, thus, present can hinder the accurate diagnosis of a disease, especially if one type of dehiscence is associated with another type. To the best of our knowledge, this is the first reported case of a cochlear–internal auditory canal dehiscence associated with a cochlear–facial dehiscence.

CASE PRESENTATION
A 53-year-old patient with the diagnosis of a refractory bilateral Meniere’s disease was referred to our center. He recalled that symptoms started with hearing loss fluctuation in the right ear after a head trauma 6 years ago; subsequently, the patient began experiencing vertigo spells, drop attacks, and tinnitus. During the last 12 months, fluctuating hearing loss associated with severe vertigo spells and tinnitus developed in the left ear as well. Thus, the patient was diagnosed with Meniere’s disease according to the clinical American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) criteria [3].

He was previously treated with betahistine, acetazolamide, and intratympanic steroids in the left ear without any clinical improvement. Physical examination yielded the following: normal otoneurological findings and normal vestibular evoked myogenic potentials. Furthermore, video head impulse test showed normal gain for all semicircular canals except for both posterior canals. Mean tone audiometry revealed a moderate sensorineural hearing loss on the right ear (55 dB) and a severe sensorineural hearing loss on the right side (80 dB). The speech discrimination score was 84% on the left side and 68% on the right side. Brain magnetic resonance imaging (MRI) performed at another institution showed normal findings (Figure 1). Hearing loss was managed with a cochlear implant (CI) on the right ear, and vertigo spells were managed with intratympanic gentamicin injections in the left ear.

Surgery was started following the standard CI procedures through posterior tympanotomy; however, due to bulging of the sigmoid sinus and a prolapsed tegmen, a canal wall-down mastoidectomy with an external auditory canal closure was performed. Surprisingly, the round window membrane was found to be distended prior to its manipulation and when punctured, a gusher of cerebrospinal fluid (CSF) was immediately noticed; it lasted over 15 min. Later, a Nucleus slim straight electrode (Cochlear, Aus-
tralia) surrounded by the fascia in the base was inserted. There was no suction or entry of blood directly into the inner ear, and no resistance was perceived during the insertion. Thus, the presence of the gusher when opening the round window before the electrode array insertion excludes the possibility of a fistula created during the procedure. The electrophysiological tests (impedances and NRT threshold) showed values in normal ranges, and the intraoperative radiological control was adequate. The round window was then sealed with fascia; the leakage of CSF was controlled, and finally, the cavity was filled with surrounding muscle tissue and fibrin glue. In order to determine the cause of the gusher, computed tomography (CT) was performed the following day (Siemens Somatom Definition 1 mm slice thickness/0.7 slice increment Axial). A dehiscence affecting the cochlear-internal auditory canal and facial canal on the right side was found; there were no signs of temporal bone fracture, and the left side showed no abnormalities (Figure 2). Regarding the cochlear aqueduct, only the initial portion was visible, without dilatation or patency of the distal part (not shown on images). The disyllabic word test recognition for speech discrimination performed 5 months postoperatively was 72%. Vertigo spells stopped after the intratympanic gentamicin injections in the left ear. No facial stimulation or spasms were observed after implant activation. Informed consent was obtained from the patient.

**DISCUSSION**

The presence of dehiscence in the otic capsule can be an acquired syndrome when associated with pathological conditions (tumors or cholesteatomas), elevated intracranial pressure (as described in tegmen thinning), or an age-related mechanism causing otic capsule bone resorption [4]. On the other hand, this entity may be congenital, caused by an alteration in the embryologic development of the otic capsule such as cases of cochlear-internal auditory canal dehiscence in Mondini-like dysplasia or cochlear-facial dehiscence associated with Fallopian canal dehiscence. The fact that the cochlea and vestibule show no malformations suggests that the defect develops at a stage of development that is more advanced than the otocyst stage [5]. The labyrinthine capsule remains cartilaginous until the inner ear reaches its final size at about 25 weeks of gestation. The interscalar septum and the bone between the cochlea and the vestibule develop from the ossification centers. Partly missing this bone suggests over-resorption, resulting in a defect of the cartilaginous capsule and a failure of ossification [6]. In our patient, presence of an otic capsule dehiscence at two levels, internal canal and facial nerve, suggests that it may be a congenital disorder rather than an acquired one related to the history of head trauma. The fact that CT was performed postoperatively may bring up the possibility of such dehiscence being an artifact. Yet, as it is clearly shown in the images (Figure 2 a, c), the study was clear of metal-related artifacts, and as can be noted on the opposite side of the cochlea, the implant itself does not impede the evaluation of the adjacent bone. Also, we have not found any similar image on CT scans of other patients after cochlear implantation (not shown).

An otic capsule dehiscence opens up a third window into the inner ear, and clinical findings such as a low-frequency bone conduction hearing loss, dizziness, vertigo, or Tullio phenomenon have been reported [2,7]. When presuming an otic capsule dehiscence, it is important to consider other conditions that might resemble or mimic a third window syndrome, as is the case of vestibular aqueduct enlargement or perilymphatic fistula. The first one is often associated with a fluctuating and progressive neurosensorial hearing loss, and in some cases, an air-bone gap in low-frequencies similar to a third window may appear; however, its underlying mechanism is still controversial [8]. A perilymphatic fistula usually occurs after an identifiable traumatic event, and vestibular symptoms, mainly dizziness and disequilibrium, are more common than hearing loss [9].

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**Figure 1.** Axial heavily T2-weighted thin-slab maximum intensity projection MR image of the right inner ear showing normal anatomy without visible malformations or other anomalies.

**Figure 2. a-d.** Axial CT zoomed-in view of both inner ears at the origin of the facial canal (lower row: c, d) and immediately above (upper row: a, b). In the right inner ear (a, c) an osseous defect with dehiscence can be seen between the basal turn of the cochlea and the internal auditory canal (a) and between the basal turn of the cochlea and the origin of the facial canal (c). Also, note the correct location of the cochlear implant. In the left inner ear (b, d), a normal anatomy is observed, with an osseous wall between the cochlea and the internal auditory canal (b) and the facial canal (d).
In our patient, the clinical symptoms started in the right ear as a low-frequency mixed hearing loss that fluctuated and that was associated with vertigo spells. Clinical symptoms suggested Meniere’s disease. However, symptoms and the disease persisted despite medical and intratympanic treatments. As in other reported cases of otic capsule dehiscence, VEMPs were preserved [7]. Although VEMPs are highly sensitive and specific for SSCD [10], the negative finding in this scenario may be attributed to the small size of the dehiscence [2]. Unfortunately, we do not know for certain the exact role that this entity plays in the present case; could this dehiscence be the cause of the refractive treatment in this particular case of bilateral Meniere’s disease? Was the right ear more affected because of it? To be able to answer the above questions, we need to become aware of and better understand this entity.

An abnormal communication between the subarachnoid space and the perilymphatic space is the underlying cause of a CSF gusher. The inner ear abnormality that is most often responsible of this phenomenon is a bony defect in the fundus of the internal acoustic meatus [11]. There are no previous reports of CSF gusher in the context of an otic capsule dehiscence, and this clinical condition may be a sign of dehiscence, as it was in the present case. In our patient, a total electrode insertion was possible and all electrodes were activated.

We present a case of a previously undescribed entity, an otic capsule dehiscence at two levels (cochlear–internal canal and cochlear–facial dehiscence) in the context of bilateral Meniere’s disease, which was responsible for a CSF gusher during a CI surgery. An accurate diagnosis of otic capsule dehiscence at two levels (cochlear–internal canal and cochlear–facial dehiscence) in the context of bilateral Meniere’s disease, which was responsible for a CSF gusher during a CI surgery. An accurate diagnosis of otic capsule dehiscence can only be made using a high-resolution temporal bone CT (2, 10). We did not perform preoperative CT in this patient because at our center, MRI is the imaging study that is routinely performed first in patients with the indication of a CI; CT is performed only in those cases where a malformation or other anatomical anomalies are detected. Thus, as a preoperative study, we recommend both MRI and CT scan.

Informed Consent: Written informed consent was obtained from the patient who participated in this study.

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Case Report

Intractable Otitis Media Presenting as Falsely Positive for Proteinase 3-ANCA: A Case Report

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Herein, we report a case of otitis media caused by methicillin-resistant Staphylococcus aureus (MRSA), presenting as falsely positive for proteinase 3 (PR3)-antineutrophil cytoplasmic antibodies (ANCA). A 47-year-old woman was referred to our hospital with a complaint of left otorrhea. An otorrhea culture yielded MRSA, and the patient was treated using tympanoplasty. Postoperative administration of teicoplanin lead to drug-induced neutropenia and was discontinued 4 days after the operation. One month after the operation, the patient’s otorrhea recurred, and it was accompanied by hearing impairment. The otorrhea culture yielded MRSA again, while serum was positive for PR3-ANCA (6.8 U/mL). As MRSA was detected in the patient’s otorrhea sample, she was treated with linezolid. Her symptoms then improved immediately. Although the PR3-ANCA positivity remained, the patient’s otorrhea and hearing impairment had not recurred for 3 years when this report was submitted. Therefore, we conclude that this is a case of false PR3-ANCA positivity.

KEYWORDS: Methicillin-resistant Staphylococcus aureus, granulomatosis with polyangiitis, Wegener granulomatosis, teicoplanin, linezolid

INTRODUCTION
Antineutrophil cytoplasmic antibodies (ANCAs) to myeloperoxidase (MPO) and proteinase 3 (PR3) are associated with primary vasculitis affecting small- to medium-sized vessels. Systemic vasculitis involving these antibodies is known as ANCA-associated vasculitis (AAV) [1]. The initial signs of AAV are otologic symptoms, such as otitis media, hearing loss, vertigo, and facial palsy. Nonetheless, the AAV diagnosis is often challenging when symptoms are localized to the ear [2]. For this reason, the study group of the Japan Otological Society recently proposed a new diagnosis: otitis media with AAV (OMAAV) [3]. OMAAV is classified if the following three criteria (A, B, C) are fulfilled: (A) a disease onset with initial sign/symptoms due to intractable otitis media with effusion or granulation, which was resistant to antibiotics and insertion of tympanic ventilation tubes; (B) at least one of the following three findings: (1) positivity for the serum MPO- or PR3-ANCA; (2) histopathology consistent with AAV, which is necrotizing vasculitis predominantly affecting small vessels with or without the granulomatous extravascular inflammation; and (3) at least one accompanying sign/symptom of the AAV-related involvement other than the ear (eye, nose, pharynx/larynx, lung, kidney, facial palsy, hypertrophic palychymeningitis, and others); and (C) exclusion of the other types of intractable otitis media, such as bacterial otitis media, cholesterol granuloma, cholesteatoma, malignant osteomyelitis, tuberculosis, neoplasm, and eosinophilic otitis media, as well as exclusion of other autoimmune diseases and vasculitis diseases other than AAV, such as Cogan’s syndrome and polyarteritis nodosa, among others. According to these criteria, OMAAV should only be diagnosed when the patient is positive for ANCAs or vasculitis, of which the latter is revealed during a pathological examination [3]. However, vasculitis is rarely diagnosed after the pathological examination of a head and neck lesion. Thus, positivity for ANCAs is an important finding in the diagnosis of OMAAV.

While false positivity for ANCAs occurs in ulcerative colitis, rheumatoid arthritis, systemic lupus erythematosus, infections, tuberculosis, and malignant tumor [4-6], few cases of false positivity for ANCAs have been reported in otological diseases [7]. Herein, we report a case of otitis media caused by methicillin-resistant Staphylococcus aureus (MRSA) and presenting as falsely positive for PR3-ANCA.
CASE PRESENTATION
A 47-year-old woman with a history of hypertension was referred to our hospital with a complaint of left otorrhea that had persisted for 5 years. An otoscopic examination revealed that the tympanic membrane of her left ear was perforated (Figure 1a). A sample of the otorrhea was cultured, yielding MRSA. An audiogram showed mixed hearing loss in the woman’s left ear (Figure 2a), and computed tomography (CT) revealed that the tympanic cavity was slightly clouded (Figure 3a).

Based on the diagnosis of chronic otitis media with MRSA, we performed a left tympanoplasty, with post-operative administration of teicoplanin (teicoplanin F; Fuji Pharma, Tokyo, Japan). Four days after the operation, the patient’s temperature increased, and she developed a urinary tract infection. A blood test revealed a white blood cell (WBC) count of 1600/μL, with a differential neutrophil count of 640/μL, as well as elevated C-reactive protein (CRP) levels 4.6 mg/dL (normal range 0.00–0.20 mg/dL). The woman was diagnosed with drug-induced neutropenia caused by teicoplanin, and her antibiotics were discontinued. Her fever then subsided, and her WBC and neutrophil counts were normalized for several days. However, the left otorrhea recurred 1 month after the operation. Again, hearing impairment was observed (Figure 2b), and CT revealed that the tympanic cavity was slightly clouded (Figure 3b). A tympanic ventilation tube was inserted, and the ear was irrigated. However, these treatments were ineffective (Figure 1b). MRSA was once again detected in her otorrhea, while her serum PR3-ANCA levels tested using the chemiluminescence enzyme immunoassay (CLEIA) had increased to 6.8 U/mL (normal: <3.5 U/mL). No disorders were detected in any other organs (kidneys, lungs, eye, etc.). A pathological examination of the patient’s middle ear granulation revealed non-specific inflammation. As MRSA was detected in a sample of her left otorrhea, she was administered linezolid (Zyvox; Pfizer, New York, USA) for 2 weeks, even though she was positive for PR3-ANCA. Following this treatment, the patient’s otorrhea and hearing loss quickly improved (Figure 2c). After the treatment, her PR3-ANCA levels remained mildly elevated (6.5 U/mL), although her otorrhea had not recurred for 3 years after the article was submitted. Therefore, we concluded that the patient was falsely positive for PR3-ANCA.

DISCUSSION
The typical clinical features of OMAAV, recently proposed by the Japan Otological Society, are the following: (1) intractable otitis media with...
Effusion or granulation that does not respond to antibiotics or insertion of a tympanic ventilation tube; (2) gradual hearing loss (in most cases) due to effusion and granulation in the middle ear, followed by sudden, progressive hearing loss within 2 months; (3) the MPO- or PR3-ANCA positivity (in most cases); (4) facial palsy and hypertrophic pachymeningitis (occasionally) [3]. According to the OMAAV diagnostic criteria, other disease, including intractable bacterial otitis media, must be excluded before OMAAV can be diagnosed [3]. In the present case, the otitis media was intractable, and the patient was positive for PR3-ANCA. However, MRSA was detected in her otorrhea, and neutropenia caused by teicoplanin was presumed to be one of the reasons for her intractable otitis media. In addition, linezolid administration improved her clinical symptoms immediately, and the disease did not recur, even though neither glucocorticoids nor immunosuppressants were used. Therefore, we concluded that the patient was falsely positive for PR3-ANCA.

False positivity for ANCA can occur in various diseases, such as other autoimmune diseases (ulcerative colitis, rheumatoid arthritis, systemic lupus erythematosus); infections (bacterial, fungal, tuberculosis); and malignant tumor [4-6]. In this regard, higher ANCA titers and the involvement of multiple affected organ systems may help to discriminate between AAV and other diseases in ANCA-positive patients [5]. In the cases of a low ANCA titer in which symptoms are localized to the ear, as in the present report, the possibility of false ANCA positivity should be considered.

The mechanism of false ANCA positivity is unknown. Some patients with PR3-ANCA-associated vasculitis have antibodies that react with a protein produced from PR3-antisense RNA. The amino acid sequence of this protein is partially homologous with a protein found in many microbes and viruses, including Staphylococcus aureus. Therefore, it has been speculated that such bacterial organisms mimic the peptide sequences of granule components and that this leads to the PR3-ANCA production [8]. It follows that the false ANCA positivity in the present case may have been related to a chronic MRSA infection.

Yamauchi et al. [7] reported a case of tuberculous otitis media presenting false PR3-ANCA positivity. Such intractable tuberculous otitis media does share some clinical features with MRSA infection with false ANCA positivity, as well as with OMAAV. It follows that this disease might be misdiagnosed as OMAAV, and various diseases that can yield false ANCA positivity must be excluded when diagnosing OMAAV. Conversely, Azuma et al. [9] reported a case of otitis media with MPO–ANCA being positive in which the otorrhea culture showed MRSA infection. In that case, the administration of an anti-MRSA drug was ineffective, while immunosuppressant therapy did improve the otic symptoms. The authors concluded that the otitis media was a symptom of vasculitis. In future similar cases, tentative use of antibiotics might be useful in diagnosis.

In addition, the ANCA detection method should be considered carefully. A variety of different methods have been developed to detect ANCA, such as the enzyme-linked immunosorbent assay (ELISA), capture ELISA, anchor ELISA, and CLEIA. In particular, CLEIA, which has high sensitivity and specificity, has yielded a larger number of false-positive results than ELISA [10]. In the present study, we tested ANCA using CLEIA. Therefore, it may be that this detection method influenced the results of the PR3-ANCA detection.

In Japan, the number of patients diagnosed with AAV has increased two- to threefold over the past 10 years. The number of patients with OMAAV is expected to increase accordingly. Thus, in cases of intractable otitis media, clinicians should consider the possibility of OMAAV. However, they should also exclude other diseases, even in cases of the ANCA positivity, which were mentioned as diagnostic criteria for OMAAV.

Informed Consent: Written informed consent was obtained from patient who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.O.; Design - M.O., K.S.; Supervision - N.H.; Resources - M.O., K.S.; Materials - M.O., K.S., D.T., M.T., H.Y.; Data Collection and/or Processing M.O., D.T., M.T., H.Y.; Analysis and/or Interpretation - M.O., M.T., H.Y.; Literature Search - M.O., H.O.; Writing Manuscript - M.O., H.O.; Critical Review - N.H.
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Langerhans Cell Histiocytosis of Bilateral Mastoid Cavity

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INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare disease, which may involve various organ systems; therefore, it has multiple clinical manifestations. Case report: We present the case of a 56-year-old woman admitted to Amerikan Hospital Ear-Nose and Throat outpatient clinic with a complaint of progressive hearing loss in both ears, which had started 10 years ago. She was treated with corticosteroids for 10 years until last year, 2017. Surgical exploration was performed and histologic evaluation revealed LCH. Conclusions: LCH has clinical manifestations depending on the site of infiltration. In adults, isolated bilateral mastoid infiltration, as an initial symptom, is a very rare condition. With corticosteroid uptake, the period of initial symptom was 10 years in our patient, which is, as per our knowledge, the longest reported in literature. This infiltration may mimic acute or chronic infections of the ear. Therefore, LCH should be considered in the differential diagnose of patients who present with bilateral mastoid cavity disease.

KEYWORDS: Langerhans cell, histiocytosis, mastoid

CASE PRESENTATION

A 57-year-old woman presented with hearing loss in both ears for 10 years, which worsened in the last 6 months. She had a diagnosis of rheumatoid arthritis for 11 years, which was treated with corticosteroids for 10 years. She self-stopped the usage of oral corticosteroid tablets 9 months ago. She had a history of Hashimoto's thyroiditis and regular levothyroxine tablet intake. She was a non-smoker, and her family history was unremarkable.

Upon examination, the external ear canals and tympanic membranes were edematous. There was no pain in the mastoid areas with palpation. In an audiological evaluation, the tympanograms of both ears were of type B and pure tone averages were 25 dB and 23 dB for the left and right sides, respectively, with a mild conductive component on both sides.

Computed tomography (CT) scan of the temporal bone revealed soft tissue lesions with mastoid cortex erosion in both ears (Figure 1).

Complete blood count results were in normal ranges. Exploration for the right mastoid cavity was performed. During the operation, the external ear canal was observed as partially eroded, the mastoid cortex was eroded, and the cavity was invaded with soft tissue mass; the bone overlying the sigmoid sinus and dura was also partially defective because of the same soft tissue (Figure 2). Frozen sections of this hemorrhagic soft tissue were analyzed and LCH was reported. Near-total excision of the soft tissue mass was performed without causing any injury to the anatomic structures in the mastoid cavity.

After confirmation of the persistent diagnosis, staging examinations were performed.

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A positron emission tomography scan revealed a nodular 1 × 1-cm mass in the right lung and high avidity of fluorodeoxyglucose in the second cervical vertebra, both reported as LCH infiltrations. The case was diagnosed as “multifocal LCH disease” by the medical oncology department, and systemic treatment including prednisone and vinblastine was initiated.

The patient returned to her home country after the first session of chemotherapy. The communication with the patient and her local physicians continued for 1 month and was lost thereafter.

In addition, because of the lack of communication, no informed written consent was obtained from the patient. This case report does not contain any specific identity information of the patient.

DISCUSSION

LCH is divided into three groups: acute disseminated form, chronic multifocal form, and chronic focal form. Acute disseminated form is known as the Letterer-Siwe syndrome and chronic multifocal form is known as the Hand-Schuller-Christian syndrome. The age of onset is important in the prognosis, whereas the prognosis is better in adults. Localized LCH has a good prognosis than the multiple-organ-involvement form.[4]

Our patient was an adult with the disease in both ears. Clinical manifestation and audiological findings were first interpreted as infectious diseases of the bilateral middle ear. However, the history of the aggravation of the symptoms after discontinuing corticosteroids lead us to anticipate a different pathology than an ordinary otitis media. In literature, most reported cases with temporal involvement experienced ear symptoms for shorter periods than did our patient.[5, 6] The regular use of corticosteroid may be the reason for the patient to have mild problems of the ears and lengthen the period of the disease to become evident.

Granuloma formation in different organ systems causes different clinical manifestations; consequently, the misdiagnosis rates are very high. Definitive diagnosis can be achieved only with the detection of CD1a and S-100 proteins and Birbeck granules adhering to the cytoplasmic membrane.

Generally, we do not use frozen section examination during acute or chronic otitis media surgeries. However, in our patient, the history, bilateral involvement of mastoid bones, patient’s hearing level changes after corticosteroid discontinuation, and different appearance of the soft tissue under operating microscope compared to inflammation or cholesteatoma led to perform a frozen section evaluation with the suspicion of LCH. Thereafter, with the diagnosis of the LCH, the soft tissue excision was performed with extreme caution without injuring the normal anatomical structures. In addition, triamcinolone-soaked gel foam pieces were applied to the cavity at the end of the operation.

In mastoid operations planned for LCH infiltration, a conservative surgery is recommended. Modugno et al. also underlined that the ossicular chain may remain uninvolved, which makes conservative surgical treatment possible.[6]

After the definitive diagnosis, staging examinations revealed lung and rib involvements in our patient; however, there are also some cases with only temporal bone involvement reported in literature. Kleinjung et al. [7] reported an LCH case in a 2-year-old child with only bilateral mastoid infiltration. Bone involvements are seen mostly in skull bones; femur, pelvis, or vertebra involvements are rare. Temporal bone involvement leads to symptoms such as hearing loss, otalgia, retroauricular swelling, otorrhea, and ear discharge, which may mimic the symptoms of otitis media. In magnetic resonance imaging and CT, osteolytic lesions and soft tissue masses are usually seen.

Chemotherapy, radiotherapy, and surgery are treatment options for LCH. Localization of the tumor is an important factor for choosing the treatment regime. Chemotherapy is the treatment option for a multifocal disease, radiotherapy for lesions that cause organ dysfunction, and corticosteroids for local lesions.[8]
In conclusion, LCH is a rare, idiopathic disease involving all systems. Therefore, otolaryngologists should consider granulomatous diseases and LCH in the differential diagnose of bilateral mastoid diseases.

CONCLUSION
LCH with only ear complaints in adults is a rare condition. In contrast, particularly in bilateral mastoid diseases without an onset period of the complaints, LCH should be kept in mind to avoid misdiagnoses and over treatment.

Informed Consent: Written informed consent could not be taken from the patient, because of the lack of communication. This case report does not contain any identity information about the patient.

Peer-review: Externally peer-reviewed.


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Letter to Editor

Comments on Tao et al. (2017), “Multiple-Frequency Matching Treatment Strategy for Tinnitus”

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Dear Editor

The article “Multiple-Frequency Matching Treatment Strategy for Tinnitus” by Tao et al. [1] provides pilot study outcomes for a sound-based tinnitus therapy, and it was published in 2017 in the Journal of International Advanced Otology. The consolidated Standards of Reporting Trials (CONSORT) statement [2], endorsed by this journal, recommends that research be reported in a manner that supports transparency and future reproducibility. Accordingly, our reading of this publication revealed several fundamental methodological issues and factual errors. Our concerns regard potentially inaccurate study conclusions, which are likely the result of unclear study rationale and methodology reporting.

Study aims: The study aim in the abstract has two parts, as stated by the authors: (1) to integrate commonly used tinnitus measures into a comprehensive questionnaire; and (2) studying the effectiveness of a masking therapy based on multiple-frequency matching for tinnitus sufferers. The first aim highlighted in this study is not appropriate for the purpose of measuring the outcome of an intervention, as it is not common to cherry pick the items from standardized outcome measures and to create a composite scale as this may result in biased and misleading results [3, 4]. Also, the second aim is not supported by an appropriate study design. For instance, with a limited sample size and other issues highlighted in this letter, the study reported is a pilot or feasibility outcome of efficacy, not effectiveness. Please refer to a report by Singel et al. [5] for the difference between efficacy and effectiveness trials. For these reasons, the study does not appear to address both the stated aims fully.

Study design: The authors report that they used a cross-over design, although it is not clear how this was conducted. There were 30 participants in this study. Without any power or sample size calculations provided, the validity of the statistical results is questioned. How randomization took place is also not clear. Authors state that the patients diagnosed with “nervous tinnitus” were included into the study. An explanation of what nervous tinnitus is and how this diagnosis was reached is lacking. It was stated that tinnitus had to be present for 12 months. However, Table 1 indicates that the disease duration was 6 months for some participants. It thus appears as though there are some contradictions. It was stated that patients underwent pure-tone and acoustic immittance audiometric examinations to characterize tinnitus. How these approaches were used to characterize tinnitus is also unclear. The degree of hearing loss the participants had was not stated, and how variations of degree of hearing loss were addressed during the sound presentation was not explained.

Treatment rationale and specifics of the signal: There was no background information about existing research regarding sound therapy or results of systematic reviews provided. There is no clear context or rationale for this study, or why a single sound was selected as the comparator. Moreover, it is not clear how “multiple-frequency masking” was actually delivered, that is, whether via an ear-level device or via the soundfield. MATLAB was mentioned in the introduction, but there was no further mention regarding how this was implemented. Was the treatment at a hospital or at home? The statement “tinnitus treatment will begin according to the result of scale” provides no clear explanation. It sounds as if tinnitus was masked; however, masking tinnitus completely is gen-

*We have reached out to the authors for their response to these comments but have not received a reply yet.

erally not advised. It would be helpful to have a rationale as to why the tinnitus was masked and not partially masked. It is alarming that there were adverse reactions such as nausea, dizziness, and hearing loss. It would be helpful to have reported how many patients had each of these symptoms. A treatment requiring patients to attend a hospital appointment for 20 minutes twice a day does not seem feasible. The time gap between treatments was not explained. The population actually selected may have been skewed to those able to attend such appointments. As there was no information on the ratio of people invited and those who were actually participating, drawing conclusions is difficult.

Outcome measures and data analysis: As highlighted earlier, it is not recommended to create a composite scale by cherry picking some items from standardized outcome measures [3, 4]. Moreover, there is great concern regarding the way the outcome measures were scored and used. The scoring of the Tinnitus Handicap Inventory is incorrect, which could invalidate all the findings in this study. Stating “never” was scored at 4, and “always” at 0, indicates that the scoring has been incorrectly reversed. For the Anxiety Scale, two of the categories mentioned are the same “most of the time” for Options 3 and 4, which is a further inaccuracy. Questions from different questionnaires were combined to make a new questionnaire. There appears to be some validity testing done on the questions extracted from the various outcome measures. As there is no mention of how this was done in the methods section, it brings the validity of this newly designed outcome measure into question. It would have been much more helpful to have a statistical analysis plan in the methods section instead of a list of what SPPS is able to do.

Factual and grammatical errors: The manuscript has many grammatical and factual errors, although we highlight only a few. The introduction contains misleading statements such as “tinnitus is an auditory nerve disorder.” There are many causes of tinnitus[6], and this is only one. The statement “tinnitus sounds usually contain three to five dominant frequencies” has no reference and would not be accurate in view of the heterogeneous nature of tinnitus. A further statement, “it is convenient to use MATLAB platform to treat tinnitus,” lacks clear rationale, and it is of questionable clinical value as not many tinnitus management providers have access to MATLAB (developed by MathWorks, Natick, United States). As such, the MATLAB application is not regarded as a convenient or conventional intervention.

Interpretation of results: In view of these limitations, stating that multiple-frequency masking is superior should be made with caution, as a significant difference was only found in the third course of treatment. The size of this difference is unknown as effect sizes were not calculated.

Overall, the reported details regarding the methodology and results are inadequate and do not logically support the authors’ conclusion regarding the efficacy of their approach. The challenges facing patients and researchers in the field of tinnitus intervention are substantial and are most adequately served when best practices regarding study design, using validated outcome measures, and interpreting results are employed. The availability of documents outlining and supporting such practices, referenced herein, merit attention from all prospective researchers and authors.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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Erratum

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In the review article by Kania et al., entitled “EAONO position statement on Vestibular Schwannoma: Imaging Assessment Question: How should growth of Vestibular Schwannoma be defined?” (J Int Adv Otol 2018; 14(1): 90-4; DOI: 10.5152/iao.2018.5360) that was published in the April 2018 issue of The Journal of International Advanced Otology, the presence of an error in the “ORCID IDs of the authors” section was detected. The error has since been corrected and the updated version of the article is available on The Journal of International Advanced Otology’s web page.
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References:

* Internal calculations based IMS Health data - IMS Health Analytics Link MAT6/2017.
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References

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