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A 3-D Analysis of the Protympanum in Human Temporal Bones with Chronic Ear Disease

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Abstract

Eustachian tube dysfunction is believed to be an important factor to cholesteatoma development and recurrence of disease after surgical treatment. Although many studies have described prognostic factors, evaluation methods, or surgical techniques for Eustachian tube dysfunction, they relied on the soft tissues of its structure; little is known about its bony structure – the protympanum – which connects the Eustachian tube to the tympanic cavity, and can also be affected by several inflammatory conditions, both from the middle ear or from the nasopharynx.

We studied temporal bones from patients with cholesteatoma, chronic otitis media (with and without retraction pockets), purulent otitis media, and non-diseased ears, looking for differences between the volume of the protympanum, the diameter of the Eustachian tube isthmus, and the distance between the anterior tympanic annulus and the promontory. Light microscopy and 3-D reconstruction software were used for the measurements.

We observed a decrease of volume in the lumen of the four middle ear diseased ears compared to the control group. We observed a significant decrease in the volume of the protympanic space in the cholesteatoma group compared to the chronic otitis media group. We also observed a decrease in the bony space (protympanum space) in cholesteatoma, chronic otitis media with retraction pockets, and purulent otitis media compared to the control group.

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We found a correlation in middle ear diseases and a decrease in the middle ear space. Our findings may suggest that a smaller bony volume in the protympanic area may trigger middle ear dysventilation problems.

INTRODUCTION

One of the main etiological factors in cholesteatoma development is long-term Eustachian tube dysfunction [1–3]. The anterior wall of the tympanum funnels into the tympanic orifice of the auditory tube. Then, it descends downward medially and anteriorly to the nasopharynx [4]. The protympanum is the bony part of the auditory tube, and it is the only opening of the tympanic cavity connecting to the nasopharynx [5]. Tóth et al. [5] showed that the main structure of the protympanum develops from the petrous part of the temporal bone while the tympanic part forms the posterior border of the protympanum laterally. It is also the location of the narrowest portion of the Eustachian tube, the isthmus [6, 7].

Although the macroscopic anatomy has been described surgically [8–11], data on the volumetric status of this area are scarcely available; the only information in the literature on this regard, to our knowledge, refers to tomographic changes among diseased middle ears [6, 12–14]. A better comprehension on its volumetric changes when comparing different types of otitis media may help to understand how the dysventilation process happens, and how those changes can lead to chronic inflammatory and intractable variations in the middle ear possible.

OBJECTIVES

The aim of this study is to evaluate the volume of the osseous portion of the Eustachian tube, both bony and mucosal volume, as well as its lumen, at the protympanic space, in ears with different types of otitis media.

MATERIALS AND METHODS

Temporal bones for this study were obtained at the Otopathology Laboratory from the Department of Otolaryngology, University of Minnesota, Minneapolis, Minnesota. The bones had been previously harvested during autopsy, fixed in 10% buffered formalin, decalcified with ethylenediaminetetraacetic acid (EDTA), dehydrated in graded concentrations of alcohol, and embedded in celloidin. Each temporal bone was serially sectioned in the horizontal plane at a thickness of 20 µm. Every 10th section was stained with hematoxylin and eosin, then mounted on a glass slide for histological study under light microscopy at various magnifications.

Samples

The *cholesteatoma group* in our study included temporal bones from patients with a diagnosis of cholesteatoma (clinically, with a history of chronic drainage, tympanic membrane perforation, and granulation tissue in the ear canal and middle ear; histologically, with the presence of a keratinized squamous epithelium in the middle ear space). The *chronic otitis media (COM) group* was selected based on histological evaluation revealing

the presence of intractable tissue changes such as granulation tissue, cholesterol granuloma, and/or bony changes within the middle ear cleft, with or without effusion in the middle ear cleft, and without cholesteatoma. The *chronic otitis media with retraction pockets (RP) group* had similar characteristics as the *COM group* with the addition of any inward displacement of the tympanic membrane from the normal position. For this evaluation, we used the classification proposed by Sadé [15], shown below:

Grade I: mild tympanic membrane retraction;

Grade II: tympanic membrane retraction in contact with incus or stapes (tympanoincudopexy);

Grade III: tympanic membrane in contact with promontory wall (not adhered to it); and

Grade IV: tympanic membrane adhered to promontory (adhesive otitis media).

The *purulent otitis media (POM) group* included temporal bones from patients with a massive number of inflammatory cells, mainly polymorphonuclears in the effusion with concomitant inflammatory infiltration, congestion, edema, and thickening of the subepithelial space without chronic tissue changes, such as the aforementioned for the *COM group*. The *control group* included 17 temporal bones from patients without any sign of ear disease. Their mean age was 37.76 ± 18.32 (range, 11 to 63 years).

We excluded temporal bones from patients with history of otologic surgeries, congenital malformation of the middle ear, temporal bone fractures, Paget disease, otosclerosis, congenital cholesteatoma, irradiation of the head and neck, infiltration by a tumor, leukemia with infiltration of the mucosa covering of the middle ear space, systemic autoimmune disease, or temporal bones affected by processing artifacts. After applying our rigorous exclusion criteria, our final *cholesteatoma group* included 6 temporal bones, mean age 63.83 ± 28.65 (range, 17 to 94 years). The *chronic otitis media with retraction pockets group* included 8 temporal bones, mean age 41.75 ± 27.4 (range, 7 to 84 years). The final *chronic otitis media group* was composed of 16 temporal bones, mean age 36.46 ± 26.76 (range, 3.5 to 74 years). The *purulent otitis media group* included 10 temporal bones, mean age 36.25 ± 29.08 (range, 0.5 to 73 years).

Middle Ear and Isthmus Measurements

To measure the lumen in the isthmus of the Eustachian tube, we used the same slide containing the umbo of the malleus. To analyze the opening of the Eustachian tube in its narrowest portion, we drew a perpendicular line to the Eustachian tube axis in its narrowest portion (e.g. isthmus). Using this line, the first measurement was from the edge of the lateral cartilage to the edge of the medial cartilage of the auditory tube (isthmus opening). The second measurement was done similarly, but only between the edges of the mucosa covering the tubal cartilage, which we refer to as the isthmus lumen (Fig. 1).

To compare the size of the middle ear cleft, we selected the last slide containing the malleus from each of the temporal bones. To analyze the protympanic space, we considered a straight line from the anterior tympanic annulus to the basal turn of the cochlea. Using this

line, we took two measurements: one from the bony part of the anterior tympanic annulus to the bony part of the promontory (protympanum opening); the second measurement was made from the edge of the mucosa covering the annulus to the edge of the mucosa lining the promontory (tympanic orifice [12]) (Fig. 1). There is no true line of demarcation between the middle ear cavity and the auditory tube, as both are formed from the tubotympanic recess or primary tympanic cavity, which communicates with the foregut [16].

A Nikon Eclipse E400 with camera Nikon DS-Ri1, Nikon, Japan equipped with measurement software (NIS-Elements BR 4.30.01 64-bit, Nikon, Japan) was used to complete the measurements.

Volumetric Analysis of the Protympanum

To scan the selected slides, we used a high-resolution scanner (PathScan Enabler IV; Meyer Instruments, Houston, TX, USA). We transferred the resulting images to 3-D reconstruction software (Amira 3D Software for Life Sciences, FEI, Hillsboro, OR, USA), and generated a 3-D model of the protympanum.

To measure the volume, we examined the slides from the first section containing the tympanic membrane to the last section containing the basal turn of the cochlea. The anterior limit was defined as the natural limit of the protympanum and, when the Eustachian tube was present, its isthmus was considered the end point. Temporal bones with no Eustachian tube available were excluded. We used the same straight line (protympanum line) from the anterior annulus of the tympanic membrane to the basal turn of the cochlea to define the posterior limit of our samples. We selected the mucosal area, to generate the mucosal volume, and the luminal area to generate the luminal volume. To calculate the volume of the protympanum, we used the sum of the measurements from each compartment.

The volume of the protympanum (Fig. 2) was defined as the sum of its lumen and its mucosa. We could not consider the entire length of the Eustachian tube, because the entire cartilaginous portion is not included during the removal process.

To calculate the volume of the protympanum of each temporal bone, we used the “Material Statistics” feature of the Amira software.

Statistical Analysis

Results are presented as the mean $\pm$ standard deviation (SD). To confirm the normal distribution of data in each group, we used the Kolmogorov-Smirnov test ($P=0.200$). For the data normally distributed, the independent t test was used, and the Mann-Whitney U test was used for those data not normally distributed. We used the software SPSS 23.0 for Windows (SPSS Inc., Chicago, IL) for statistical analysis. Findings were considered statistically significant when P values were less than 0.05.

RESULTS

Fifty-seven temporal bones, from 44 donors were included in this study. Thirty-seven temporal bones were from males and 20 temporal bones were from females. Five of the

temporal bones included in the *RP group* were classified as grade I, two temporal bones were classified as grade II, and one temporal bone as grade III (Table 1). There were no significant differences between male and female patients of middle ear measurements and volume, or for the chronic otitis media with retraction pockets based on Sade's classification.

Isthmus Opening

The mean diameter of the isthmus opening in the *chronic otitis media with retraction pockets group* was smaller than all other groups. The mean isthmus opening in the *control group* was the largest among all groups ($527.35 \pm 201.25 \mu\text{m}$).

When we analyzed the isthmus lumen only, the *POM group* showed the smallest measurements ($263.84 \pm 213.53 \mu\text{m}$, mean). However, this decrease was not statistically significant when compared to the *control group* ($P = 0.071$) (linear measurements are shown in table 2).

Protympanum Opening

The protympanum opening was similar when comparing the *COM group* to the *RP group* (3776.45 ± 674.97 vs $3778.38 \pm 983.15 \mu\text{m}$). Although we observed a decrease in the protympanum opening in the other 4 groups (cholesteatoma, chronic otitis media with retraction pockets, chronic otitis media, and purulent otitis media) compared to the *control group*, this was significant only when comparing COM to POM groups, and POM to control groups ($P = 0.045$ and $P = 0.024$, respectively).

There was a statistically significant decreased mean value of the tympanic orifice in the *cholesteatoma group* compared to the *chronic otitis media with retraction pockets group* ($P = 0.038$), the *chronic otitis media group* ($P = 0.002$), and the *control group* ($P < 0.001$). The mean value in the *cholesteatoma group* was the smallest among all groups. There was a decrease in the mean values among temporal bones in the chronic otitis media with retraction pockets, chronic otitis media, and purulent otitis media groups, but this decrease was significant only when comparing the *POM group* to the *control group* ($P = 0.013$) (Table 2).

Protympanum Volume

There was an increase in the volume of the mucosa covering the protympanum between all 4 diseased groups compared to the *control group*. The *control group* showed the smallest volume of mucosa covering the protympanic space ($23.06 \pm 8.48 \text{ mm}^3$). Interestingly, none of the four middle ear diseased groups showed a statistically significant increase in their mucosal volumes compared to the *control group* (cholesteatoma, $P = 0.093$; RP, $P = 0.428$; COM, $P = 0.121$; and POM, $P = 0.097$).

The luminal volume in the *cholesteatoma group* ($18.5 \pm 19.37 \text{ mm}^3$) was significantly decreased compared to all other groups. The volume of the protympanum in the group with cholesteatoma was the smallest among all groups ($53.94 \pm 22.63 \text{ mm}^3$). It was significantly

decreased compared to the *COM group* ($P = 0.018$), and to the *control group* ($P = 0.004$) (volumetric measurements are shown in table 3).

DISCUSSION

In this study, we found a significant volumetric reduction of the protympanic lumen in temporal bones with cholesteatoma compared to the other three middle ear diseased groups and to the *control group*. Only one previous study reported the protympanic space, as a site of obstruction, using small fiber-optic scopes introduced during surgery [8]. They found that this area was the most common site of obstruction in patients that underwent surgery for chronic ear disease (chronic suppurative otitis media, adhesive otitis media, or cholesteatoma), however they found no differences between the 3 groups [8]. Their results indicated that much of the obstruction existed in the protympanic segment of the Eustachian tube. Indeed, our study, by objective measurements, also supports the evidence concerning the protympanum status in patients with chronic ear diseases and its implications for surgical intervention and outcomes.

Sadé and Luntz [17], in a temporal bone study, evaluated the cross-sectional area of the Eustachian tube among patients with acute or secretory otitis media and non-diseased ears. They found a reduced lumen (not statistically significant) among inflamed ears compared to controls. Conticello et al. [18] described a constricted and obstructed Eustachian tube in patients with chronic inflammation of the middle ear by tomographic evaluation. We observed a constricted protympanic space (due to a narrow bony space or to a thicker mucosa) among the four middle ear diseased groups compared to the *control group*. Sudo et al. [12] studied the Eustachian tube by computed tomographic imaging in 9 temporal bones, from 9 patients with no congenital anomalies or history of ear disease. They found the narrowest portion 20 mm from the pharyngeal orifice, with an area of $0.65 \pm 0.19 \text{ mm}^2$, and the tympanic margin (close to the middle ear cleft) $3.35 \pm 2.04 \text{ mm}^2$. Our study, however, is the first to compare the protympanum volume in patients with chronic otitis media. Additionally, our results show a narrower isthmus lumen and opening in middle ear diseased patients, suggesting that this portion of the Eustachian tube is actively involved in the ventilatory function of the middle ear space.

Eustachian tube dysfunction is one of the initial factors for chronic ear disease development, but not the only factor [9, 19]. Yoon et al. [20] observed obstruction of the bony portion of the Eustachian tube in 17.9% of chronic otitis media patients. It also has been suggested that Eustachian tube dysfunction may play a major role in the development of attic retraction pockets [21]. Tarabichi and Najmi [14] stated that the upper segment of the Eustachian tube, close to the tympanic cavity, is commonly exposed to the majority of childhood middle ear inflammatory diseases, making it a logical candidate for acquired inflammatory stenosis and irreversible damage to temporomastoid system. Eustachian tube dysfunction, caused by a blockage or a narrow protympanic space, may be the main factor that triggers middle ear diseases. A reduced volume of its bony segment, the protympanum, was observed in temporal bones with the middle ear diseases included in this study. Our findings support the idea of a constricted space in the middle ears of diseased patients.

It has been demonstrated that otitis media occur as a continuum of correlated diseases [22]. Obstruction of the Eustachian tube leads to progressive inflammatory changes in the middle ear cleft; if the obstruction is not resolved, it often leads, ultimately, to formation of intractable changes in the middle ear, such as granulation tissue, cholesterol granuloma, and cholesteatoma [20,22]. Jufas et al. [23] demonstrated the importance of the anatomy of the protympanum in the ventilation of the middle ear; they suggested further investigation of therapies targeting control of the inflammation and improving the ventilation pathway in that region. The findings of our study demonstrate that patients with diseased ears have a significant decrease in the volume of the protympanum when compared to patients without middle ear disease. When considering that the middle ear cleft is a bony, non-collapsible structure, the variation of the pressure of air creates positive and negative forces inside its chamber [24,25]. Several regulatory mechanisms can neutralize the variation of the pressure, including the natural air reservoir (mastoid air space) and the intermittent opening mechanism of the Eustachian tube [24,25]. Smaller compartments may create a favorable ambient to dysventilation, decreasing the ability of the middle ear cleft to deal with these variations in the gas pressure [25,26]. Furthermore, other studies also demonstrated that patients with retraction pockets and attic disease also have either decrease in the volume of selected middle-ear compartments or blockage in the ventilation pathways of the middle ear cleft [26,27,28]. Thus, clearance of that region (either by clinical or surgical treatment) may have a higher impact in the progression of those diseases, reducing the long-term sequelae that are frequent in the later stages of otitis media [22–26].

CONCLUSION

Our findings suggest that a smaller bony volume in the protympanic area may trigger middle ear dysventilation problems.

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Figure 1.

A representation of how the boundaries were delimited to calculate the protympanum volume for each different group: A = control, B = chronic otitis media, and C = cholesteatoma (H&E, 1x magnification). A straight line from the anterior tympanic annulus to the basal turn of the cochlea was the posterior limit. We defined the anterior limit as a straight line connecting the posterior limits of the Eustachian tube cartilage was the anterior limit. The gray line represents the limits of the mucosa, and the magenta line, the limits of the lumen. Note how narrow the protympanic space is among diseased ears compared to the control ear. Additionally, note how thick the mucosa is in diseased ears compared to a control case.

C = cochlea; Ch = keratinized epithelium within the middle ear (cholesteatoma); EAC = external auditory canal; FN = facial nerve; GT = granulation tissue; I = incus; ICA = internal carotid artery; M = malleus; ME = middle ear; PSSC = posterior semicircular canal; S =

stapes; TM = tympanic membrane; V = vestibule Stained with hematoxylin and eosin, 1X magnification

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Figure 2.
Representation of the 3-D reconstruction of the protympanic space. A: Anterior view. B: Posterior view from middle ear. C: Medial view.
ICA = internal carotid artery impression; TM = tympanic membrane. * = Tensor tympani muscle canal, ** = lumen

Comparison of measurements among temporal bones in the retraction pocket group (age expressed in years, distance expressed in μm).

Table 1

Age	Sade's classification	Isthmus lumen	Isthmus opening	Tympanic orifice	Protympanum opening
18	I	231.45	507.55	3032.22	3858.91
7	III	308.89	409.4	1972.29	2245.53
73	I	286.38	415.35	5184.23	5574.04
84	I	450.84	607.45	1968.65	3128.08
30	II	427.02	560.58	3137.88	3292.58
51	II	324.99	481.05	2994.38	3714.89
50	I	109.91	386.22	3464.84	3927.18
21	I	268.4	509.26	4086.83	4485.9

Table 2

Linear measurements of the protympanic space and its statistical relations between the 5 groups.

	Mean $\pm$ SD (μ m)	Cholesteatoma	RP	COM	POM	Control
Isthmus lumen						
Cholesteatoma	299.49 $\pm$ 94.85	-	.199	.212	.913	.079
RP	300.98 $\pm$ 107.96	.199	-	.700	.183	.449
COM	281.65 $\pm$ 117.08	.212	.700	-	.225	.181
POM	263.84 $\pm$ 213.53	.913	.183	.225	-	.071
Control	353.62 $\pm$ 177.22	.079	.449	.181	.071	-
Isthmus opening						
Cholesteatoma	509.59 $\pm$ 72.86	-	.170	.130	.913	.294
RP	484.6 $\pm$ 77.59	.170	-	.774	.214	.571
COM	506.21 $\pm$ 201	.130	.774	-	.225	.765
POM	484.9 $\pm$ 312.75	.913	.214	.225	-	.132
Control	527.35 $\pm$ 201.25	.294	.571	.765	.132	-
Tympanic orifice						
Cholesteatoma	2446.61 $\pm$ 1094.31	-	.038 *	.002 *	.128	<.0001 *
RP	3230.16 $\pm$ 1061.28	.038 *	-	.861	.324	.277
COM	3295.73 $\pm$ 739.01	.002 *	.861	-	.115	.202
POM	2701.26 $\pm$ 1123.75	.128	.324	.115	-	.013 *
Control	3609.81 $\pm$ 644.69	<0.0001 *	.277	.202	.013 *	-
Protympanum opening						
Cholesteatoma	3745.95 $\pm$ 263.12	-	.131	.210	.914	.141
RP	3778.38 $\pm$ 983.15	.131	-	.996	.183	.683
COM	3776.45 $\pm$ 674.97	.210	.996	-	.045 *	.471
POM	3076.99 $\pm$ 1077.8	.914	.138	.045 *	-	.024 *
Control	3996.03 $\pm$ 749.11	.141	.683	.471	.024 *	-

COM = chronic otitis media, POM = purulent otitis media, RP = chronic otitis media with retraction pockets.

statistically significant
= *

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Table 3

Volumetric measurements of the protympanic space and its statistical relations between the 5 groups.

	Mean $\pm$ SD (mm ³)	Cholesteatoma	RP	COM	POM	Control
Mucosal volume						
Cholesteatoma	35.44 $\pm$ 12.61	-	.056	.606	.159	.093
RP	30.86 $\pm$ 12.94	.056	-	.713	.859	.428
COM	31.39 $\pm$ 15.64	.606	.713	-	.874	.121
POM	27.79 $\pm$ 7.17	.159	.859	.874	-	.097
Control	23.06 $\pm$ 8.48	.093	.428	.121	.097	-
Lumen volume						
Cholesteatoma	18.5 $\pm$ 19.37	-	.034 *	.005 *	.017 *	.001 *
RP	56.58 $\pm$ 19.37	.034 *	-	.540	.989	.081
COM	63.04 $\pm$ 29.63	.005 *	.540	-	.493	.171
POM	51.34 $\pm$ 22.76	.017 *	.989	.493	-	.063
Control	76.41 $\pm$ 34.55	.001 *	.171	.171	.063	-
Protympanic volume						
Cholesteatoma	53.94 $\pm$ 22.63	-	.340	.018 *	.068	.004 *
RP	80.12 $\pm$ 21.34	.340	-	.115	.548	.065
COM	95.94 $\pm$ 37.23	.018 *	.115	-	.211	.829
POM	78.74 $\pm$ 25.22	.068	.548	.211	-	.340
Control	99.45 $\pm$ 41.08	.004 *	.065	.829	.340	-

COM = chronic otitis media, POM = purulent otitis media, RP = chronic otitis media with retraction pockets.

* = statistically significant