Evidence Against the Mucosal Traction Theory in Cholesteatoma

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**Objectives**: To investigate the distribution of ciliated epithelium in the human middle ear and its potential role in the formation of cholesteatoma.

**Study Design**: Comparative human temporal bone study.

**Methods**: We selected temporal bones from 14 donors with a diagnosis of cholesteatoma, 15 with chronic otitis media without retraction pockets, 14 with chronic otitis media with retraction pockets, 14 with cystic fibrosis (CF), and 16 controls. We mapped the distribution of the ciliated cells in the mucosal lining of the middle ear and tympanic membrane using three-dimensional reconstruction analysis, and counted the number of ciliated cells in the middle ear mucosa.

**Results**: Ciliated cells are extremely sparse in the epithelial lining of the lateral surface of the ossicles in the epitympanum and the medial surface of the tympanic membrane. Furthermore, there is a significant decrease in the number of ciliated cells in these areas in temporal bones with cholesteatoma, chronic otitis media, chronic otitis media with retraction pockets, and CF compared to controls. Ciliated cells most commonly are located at the hypotympanum and the Eustachian tube opening but not the tympanic membrane or epitympanum.

**Conclusion**: The paucity of ciliated epithelial cells on the medial side of the tympanic membrane and the lateral surface of the ossicles in the epitympanum in cases with cholesteatoma and/or chronic otitis media do not support the mucosal migration theory of cholesteatoma formation.

**Key Words**: Ciliated cells, histopathological on chronic ear diseases, cystic fibrosis, cholesteatoma, retraction pockets.

**Level of Evidence**: NA.

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**INTRODUCTION**

A cholesteatoma is a non-neoplastic epithelial lesion that contains layers of keratin in a cavity lined by keratinizing squamous epithelium and subepithelial connective tissue. Ciliated cells are extremely sparse in the epithelial lining of the lateral surface of the ossicles in the epitympanum and the medial surface of the tympanic membrane. Although benign, it can cause serious complications by eroding nearby structures or precipitating infection. Surgical removal of the lesion is considered the only effective medical therapy against the disease.

Cholesteatomas may be congenital or acquired. There are several theories regarding the etiopathogenesis of acquired cholesteatoma, such as: 1) metaplasia, 2) basal cell hyperplasia, 3) immigration through a perforation; 4) iatrogenic, posttraumatic or implantation; and 5) invagination via retraction pockets.

Recently, Jackler et al. proposed a new theory for the genesis of cholesteatoma based on the premise that a squamous pouch is drawn inward by traction exerted by the interaction of opposing ciliated epithelial surfaces of middle ear mucosa on the medial surface of the tympanic membrane and the lateral surface of the ossicles. According to these authors, if the mucosal surface of the tympanic membrane comes in contact with the mucosa of the lateral surface of the ossicles, the two mucosal surfaces become coupled. Once coupled, the ciliary beat leads to mucosal migratory propulsion of the conjoined layers, dragging the pliable tympanic membrane superorly. The authors claim that the proposed mechanism is more closely aligned with the observed characteristics of the disease than earlier theories.

The aim of this study is to evaluate the changes in the middle ear mucosa and tympanic membrane in human temporal bones, especially those with possible implications to the formation of cholesteatoma, in four pathologic conditions (cholesteatoma, chronic otitis media without cholesteatoma, retraction pockets, and cystic fibrosis [CF]) compared to nondiseased controls.

**MATERIALS AND METHODS**

Seventy-three human temporal bones for this study were obtained at the Otopathology Laboratory in the Department of Otolaryngology, University of Minnesota, Minneapolis, Minnesota, U.S.A. Immediately after removal, the temporal bones were placed...
in 10% buffered formalin. They were then decalcified, embedded in celloidin, and sectioned at a thickness of 20 μm. Every 10th section was stained with hematoxylin and eosin for histological study under light microscopy (Lily MCX500, Micros, Glan, Austria) at various magnifications (Fig. 1).

The following five groups of temporal bones were studied: 1) cholesteatoma, 2) chronic otitis media without cholesteatoma (COM), 3) retraction pockets (RPs), 4) CF, and 5) a control group with no middle ear disease. We excluded all patients who had a history of otologic surgery, congenital malformation of the ear, temporal bone fractures, Paget’s disease of bone, otosclerosis, congenital cholesteatoma, or leukemia. We also excluded temporal bones with fixation or removal artifacts, missing middle ears, or infiltration by a tumor.

The **cholesteatoma group** included temporal bones from patients with a diagnosis of cholesteatoma (a clinical history of chronic drainage with a tympanic membrane perforation, and granulation tissue in the ear canal and middle ear). The histopathological diagnosis was the presence of a keratinizing epithelium in the middle ear space. This consisted of 14 temporal bones (9 males and 5 females); mean age 57.4 ± 29.0 years (range: 7–88 years).

The **chronic otitis media group** was selected based on histological evidence 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. We excluded from this group temporal bones with retraction pockets and included those in a separate group. This included 15 temporal bones (11 males and 4 females); mean age 44.9 ± 28.0 years (range: 7–89 years).

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The **chronic otitis media with retraction pocket group** had characteristics similar to those of the COM group, with the additional evaluation of any inward displacement of the tympanic membrane from the normal position. For this evaluation, we used the classification proposed by Sadé: 1) grade I = mild tympanic membrane retraction; 2) grade II = tympanic membrane retraction in contact with the incus or stapes (tympanoincudopexy); 3) grade III = tympanic membrane in contact with the promontory wall (not adhered to it); and 4) grade IV = tympanic membrane adhered to the promontory (adhesive otitis media). This group included 14 temporal bones (11 males and 3 females); mean age 59 ± 12.08 years (range: 45–84 years).

The **cystic fibrosis group** consisted of temporal bones from patients with a clinical and laboratory (positive sweat chloride test) diagnosis of cystic fibrosis. Patients with cystic fibrosis were selected for this study due to the low incidence rates of cholesteatoma and chronic otitis media in this group. This group included 14 temporal bones (6 males and 8 females); mean age 21.0 ± 6.5 years (range: 10–27 years).

The **control group** included 16 temporal bones (from 10 males and 6 females) without any signs of ear disease. Their mean age was 54.3 ± 6.5 years (range: 1–77 years).

### Analysis of the Epitympanum and Tympanic Membrane

The medial surface of the tympanic membrane (both the pars tensa and the pars flaccida) and the lateral epitympanum, as well as its structures (the lateral surface of the incus and malleus), were analyzed under light microscopy. All ciliated cells of every stained section included in this area were counted and recorded as absolute numbers.

[Fig. 1](#) (1) Histological analysis of the pars tensa and the lateral surface of the lateral protympanum demonstrating flat epithelial cells and ciliated cells in a normal human temporal bone. (2) The arrows indicate the squamous epithelium without the presence of ciliated cells or goblet cells. (3) The arrowheads indicate the presence of ciliated cells distributed over the lateral wall of the protympanum. C = cochlea; ET = Eustachian tube; FN = facial nerve; ICA = internal carotid artery; PSCC = posterior semicircular canal; RWM = round window membrane; RWN = round window niche; TM = tympanic membrane. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]
Analysis of the Protympanum

Ciliated cells at the protympanum were counted in a single horizontal section traversing the most inferior extent of the malleus, located at the lateral wall—from the anterior tympanic annulus to the Eustachian tube isthmus—by light microscopy.

Volumetric Analysis of the Middle Ear and Tympanic Membrane

Using a high-resolution scanner (PathScan Enabler IV; Meyer Instruments, Houston, Texas, U.S.A.), and three-dimensional (3D) reconstruction software (Amira, Visualization Sciences Group, Bordeaux, France; and Zuse Institute, Berlin, Germany), we created a 3D model of the middle ear of one temporal bone in the control group as a visual example of the distribution of ciliated cells.

Statistical Analysis

Results are presented as the mean ± standard deviation. The five groups were compared together using the nonparametric Kruskal-Wallis test. If the overall test was significant (P < 0.05), the cholesteatoma group was compared to the other four groups with the nonparametric Wilcoxon rank sum test, after applying the Bonferroni correction for multiple comparisons (P < 0.05/4 = 0.0125). Correlations between the severity of the groups and the number of ciliated cells in the three areas of the middle ear were analyzed in the following sequence: control, CF, COM, RP, and cholesteatoma. This order has been defined in observations of pathologic changes to the middle ear structures reported by previous studies. Temporal bones from donors who had CF usually do not have pathologic changes in the middle ear cleft.5 Regarding the COM groups, studies have reported increased degrees of dysventilation to the epitympanum in temporal bones with RPs than ears without RPs; thus, COM bones with RPs are included in a higher severity degree than COM without RPs.10 The presence of cholesteatoma is the ultimate stage of the continuum of otitis media. It is associated with more severe destruction and mucosal changes in the middle ear cleft, features that are less frequently observed in cases of COM without cholesteatoma.11 To observe the correlation between the number of ciliated cells in the mucosa of the epitympanum and the severity of the disease, we used the nonparametric Spearman correlations test. All statistical analyses were performed using SPSS 23.0 for Windows (SPSS Inc., Chicago, Illinois, U.S.A.).

RESULTS

Epitympanum

We did not find differences in the number or distribution of ciliated cells between the pars tensa and pars flaccida of the tympanic membrane among the groups. Although ciliated cells were rare in the epitympanum in all groups, the mean number of ciliated cells at the mucosal lining of the lateral epitympanum and lateral surface of the malleus and incus were significantly reduced in human temporal bones with cholesteatoma when compared to all other groups (P < 0.0125). There was a reduction in the number of ciliated cells located at the epitympanum in the RP group compared to nondiseased ears (Table I). When present, the ciliated cells were most commonly located at the lateral wall of the epitympanum, not on the ossicles. The nonparametric Spearman correlation was −0.49 (P < 0.001) for the epitympanum.

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<th>TABLE I. Absolute Value of Ciliated Cells in the Epitympanic Area, Tympanic Membrane, and Lateral Wall of the Protympanic Space</th>
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*P < 0.0125.

**CF = cystic fibrosis; Chol = cholesteatoma; COM = chronic otitis media; K-W = Kruskal-Wallis test; min/max = minimum/maximum; RP = retraction pockets; SD = standard deviation; stat sig = statistically significant.

Tympanic Membrane

The mean number of ciliated cells in the tympanic membrane was decreased in human temporal bones in the cholesteatoma compared to RP groups (Table I). The Spearman correlation was −0.09 (P = 0.465).

Protympanum

Ciliated cells were plentiful in the protympanum of all four of the conditions studied (Table I). The mean number of ciliated cells in the lateral wall of the protympanic space (near the Eustachian tube orifice) was decreased in human temporal bones with cholesteatoma and COM compared to the RP, CF, and control groups (Table I); and the Spearman correlation for the protympanum was −0.11 (not significant).

Some ciliated cells were found at the anterior annulus of the tympanic membrane and at the posterior portion of the handle of the malleus, in contact with the tympanic membrane (Fig. 2). The epithelium covering the lateral face of the ossicles at the epitympanum consisted of flat simple squamous epithelium, as was the epithelium of the medial surface of the tympanic membrane (Fig. 3).

DISCUSSION

The mucosa of the posterior and superior middle ear is covered by simple squamous or cuboidal epithelium. The anteroinferior half to two-thirds, extending from the Eustachian tube orifice to the hypotympanum,
**Fig. 2.** Normal distribution of ciliated cells in a three-dimensional model of the human middle ear. Medial view (superior). Lateral view (inferior). Note that most of the cells are concentrated at the Eustachian tube orifice (anterior portion marked with *), especially at the lateral wall. The epithelium covering this structure is respiratory-like. Only a few isolated ciliated cells can be seen on the ossicles and tympanic membrane.

1 = ciliated epithelium; 2 = incus; 3 = stapes; 4 = round window niche; 5 = Fallopian canal with the facial nerve; 6 = internal carotid artery; 7 = Eustachian tube cartilage; brown = ossicles; gold = tympanic membrane; gray = bony walls of the middle ear; orange = ciliated cells; red = internal carotid artery; yellow = facial nerve; A = anterior; P = posterior. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

**Fig. 3.** Histological analysis of the middle ear space. (A) Slide from a left ear, at the level of the pars flaccida, showing the space where the ciliated cells were counted at the epitympanum: lateral side of the malleus and incus, and medial side of the lateral wall. The light blue area represents the area where the ciliated cells were counted (H&E, 1 × magnification). (B) The arrow shows the flat epithelium located at the lateral side of the malleus (H&E, 10 × magnification). (C) The arrow shows the flat epithelium located at the medial side of the lateral wall at the epitympanum (H&E, 10 × magnification). (D) Histological view of the respiratory-like epithelium that covers the lateral wall of the protympanum toward the Eustachian tube. The arrowheads show the goblet cells located at this space (H&E, 40 × magnification).

CP = cochleariform process; EAC = external auditory canal; FN = facial nerve; H&E = hematoxylin and eosin; HSCC = horizontal semicircular canal; I = incus; M = malleus; TM = tympanic membrane. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]
promontory, and anterior epitympanum, are lined by pseudostratified columnar ciliated epithelium.\textsuperscript{12,13} Over the medial wall, the density of ciliated cells diminishes from the Eustachian tube to the promontory.\textsuperscript{14} The density of ciliated cells can be over 80\% of the epithelium lining the Eustachian tube, 11\% to 50\% over the promontory, and less than 1\% in the epitympanum and mastoid air cells.\textsuperscript{15} The medial side of the tympanic membrane is covered by flat, simple squamous epithelium,\textsuperscript{15,16} as observed in our study. The significant Spearman correlations for the epitympanum indicates a moderate negative association between the number of ciliated cells in the epitympanum and an increasing severity of the diagnostic groups (i.e., the more severe the diagnostic group—with irreversible and chronic changes of the middle ear cleft—the fewer the number of ciliated cells). Those findings may find support in the observation that chronic inflammation of the respiratory epithelium (trachea, in example) leads to the substitution of the cuboid cells to stratified squamous epithelium, with decrease in number and function of the ciliated cells.\textsuperscript{17}

Sharma et al.\textsuperscript{18} harvested 100 biopsies between 2008 and 2010 during surgical exploration for chronic suppurrative otitis media. They found stratified squamous epithelium covering the ossicles in 60\% of the biopsies, followed by keratinized squamous epithelium in 18\% of the cases. They also observed an incidence of 28\% of marginal perforation of the pars tensa on clinical examination and found cholesteatoma in 42.9\% of these cases, intraoperatively.\textsuperscript{18} Their conclusion, as well as those of many other studies, was that the surface of the ossicles (and the entire mastoid) is covered with simple squamous or cuboidal epithelium and not ciliated cells.\textsuperscript{15,16,19} Our study supports these previous findings because most of our specimens showed flat epithelium covering the lateral portion of the ossicles.

Several authors showed a decreased number of ciliated cells in patients with a diagnosis of acute otitis media,\textsuperscript{20–27} or chronic otitis media.\textsuperscript{28–30} Our findings (decreased number of ciliated cells in the epitympanum, lateral wall of the protympanum, and tympanic membrane of diseased ears compared to our nondiseased control group) are compatible with these studies and do not support the idea of a metaplastic change (from simple squamous to ciliated, respiratory epithelium), as proposed by Jackler et al.\textsuperscript{8} in a new theory of the genesis of acquired cholesteatoma.

CONCLUSION

In the middle ear cleft, ciliated cells are plentiful in the protympanum and hypotympanum, but they are sparse in the epitympanum and over the ossicles. In cases of chronic otitis media with and without cholesteatoma and in cases of retraction pockets, these ciliated cells were even more rare. The results of our study do not support the concept that the epithelium of the epitympanum and ossicles undergoes metaplasia to become ciliated cells. These findings do not support the mucosal traction hypothesis of the formation of acquired cholesteatoma.

BIBLIOGRAPHY