HISTOLOGICAL CHANGES OF THE MIDDLE EAR OSSICLES HARVESTED DURING CHOLESTEATOMA SURGERY

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Summary: Background: In the cholesteatoma surgery ossicles can be replaced to reconstruct middle ear function. It is important that these ossicles are free of squamous epithelium, to prevent residual disease. This study focuses on the histological findings of the malleus and incus harvested during cholesteatoma surgery. Materials and Methods: Eighty middle ears ossicles were examined in vivo and histologically to consider the relationship of cholesteatoma to ossicles, grade of bone destruction and invasion of cholesteatoma to deeper layers of bone. Results: Serious ossicular destruction was observed more frequently in incus compared to malleus ($p = 0.0065$). Difference of ossicles destruction between children and adults was not significant ($p = 0.3032$). Deep invasion of cholesteatoma into the vascular spaces or inner core of the bone was not observed. Conclusions: Autograft ossicles from cholesteatomatous ears should not necessarily be rejected for reconstruction of the ossicular chain. Regarding the histological finding, the authors suggest mechanical cleaning of the ossicle surface to eliminate residual disease.

Keywords: *Cholesteatoma; Middle ear ossicles; Incus; Malleus; Surgery*

Introduction

Autograft ossiculoplasty has been well known for more than fifty years. The first report was published by Hall and Rytzner in 1957 (1). The malleus and the incus have been used in middle ear surgery due to biocompatibility, low cost and long-term stability. However, the risk of cholesteatoma transmission limits autograft ossiculoplasty in cholesteatomatous ears. Cholesteatoma attacks middle ear ossicles in most patients. It depends on location and spreading of the cholesteatoma (Fig. 1). In these patients autologous ossiculoplasty could lead to reimplantation of cholesteatoma due to microscopic residue of squamous cell epithelium in the ossicles (2–10). Could one remove the cholesteatoma from ossicles and utilize malleus and incus for reconstruction without risk of residual disease? We studied the cholesteatoma relationship to ossicles in order to answer whether the cholesteatoma is present only on a superficial layer of the middle ear ossicle or invades deeper into the vascular spaces and inner core.

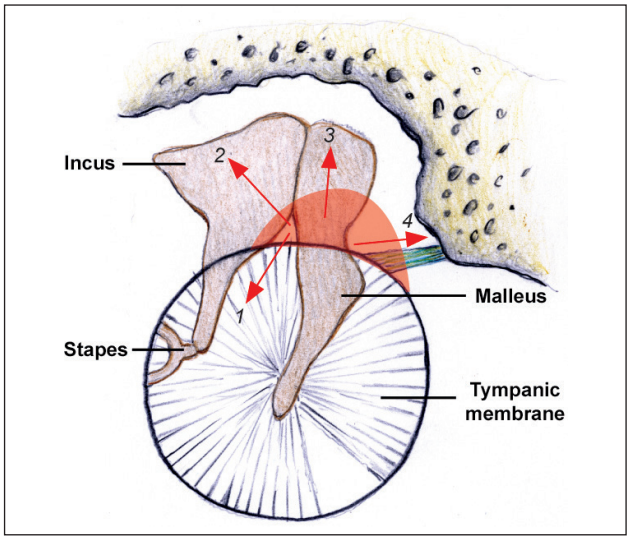

Fig. 1: Scheme of the attic cholesteatoma spreading – arrow 1 to the mesotympanum behind long process of incus, arrow 2 to the medial attic behind the body of incus, arrow 3 to the superior attic and tegmen tympani above the head of malleus, arrow 4 to the anterior attic and protympanum. (Adapted from Chrobok V et al. (20))

Materials and methods

Eighty middle ear ossicles were used for this study. As specimens, we examined mallei and incudes harvested during middle ear cholesteatoma surgery. Inclusion criteria were: chronic otitis media with cholesteatoma, primary cholesteatoma surgery, evidence of cholesteatoma on the ossicle surface. The surgeries were carried out between 2006 and 2011. The ossicles were examined and measured under microscopy. The ossicles were grouped as follows (consistent with malleus and incus erosion classification) (11):

- Ossicle destruction grade I: size of the malleus head > 2 mm, size of the incus body >3 mm in diameter.
- Ossicle destruction grade II: size of the malleus head < 2 mm, size of the incus body < 3 mm in diameter.

All specimens were fixed in 10% formaldehyde. Decalcification was performed by electrolysis in decalcifier system (SAKURA TDE™ 30 Decalcifier System, Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands). The tissue blocks were serially sectioned and stained with standard hematoxylin and eosin and examined under light microscopy.

Subjects gave written informed consent. The study was approved by our institutional Ethical Committee.

Statistical analyses

All statistical analyses were performed with SAS 9.2 (Statistical Analysis Software release 9.2, SAS Institute Inc., Cary, North Carolina, USA). The results were statistically evaluated by means of the Fisher Exact Test. A *P*-values less than 0.05 were considered to be statistically significant in all statistical analyses.

Results

Middle ear ossicles were harvested from 46 patients. There were 27 male and 19 females. Their ages ranged from 5 to 73 years with an average age of 37 years and median 43 years.

In total, 80 middle ear ossicles were histologically examined. Harvested ossicles included 43 mallei and 37 incudes. All ossicles showed evidence of cholesteatoma. Serious erosion, grade II, was observed in 24 ossicles, mild erosion grade I in 56 ossicles.

Difference of destruction between malleus and incus

Ossicular destruction grade II was observed more frequently in the incus. We found destruction grade II in 46% of incudes and only in 16% of mallei. The difference is statistically significant (p = 0.0065, Table 1). Malleus with destruction grade II accompanied incus with the same grade of destruction or complete destruction of incus.

Tab. 1: Difference of destruction between malleus and incus.

Ossicle	N	Destruction				*P*-value*
		Grade I		Grade II		
		N	%	N	%	
Incus	37	20	54.1	17	45.9	0.0065[a]
Malleus	43	36	83.7	7	16.3	
Total	80	56	70.0	24	30.0	

[a] Fisher exact test
* Difference is significant at the significance level p < 0.05.

Difference of ossicle destruction grade II between children and adults

Ossicular destruction grade II was observed in 38% of children (under 18 years of age) and 39% of adults. The difference is not statistically significant (p = 0.3032, Table 2).

Tab. 2: Difference of ossicles destruction between children and adults.

Patients	N	Destruction				*P*-value*
		Grade I		Grade II		
		N	%	N	%	
Children[1]	16	10	62.5	6	37.5	0.3032[a]
Adults[2]	28	17	60.7	11	39.3	
Total	44	27	61.4	17	38.6	

[1] ≤18 years of age
[2] >18 years of age
[a] Fisher exact test
* Difference is significant at the significance level p < 0.05.

Lymphocyte infiltration

Lymphocyte infiltration of the inner core of the ossicle was found in 5 cases, 3 malleus and 2 incudes. Statistical significance of this difference was not tested due to the small number of infiltrated ossicles.

Cholesteatoma invasion to the deeper bone layers

Cholesteatoma appeared on the surface of ossicles. In one malleus, a plug of squamous cell epithelium was also found underneath a thin bone lamella (Fig. 2). No deeper in-

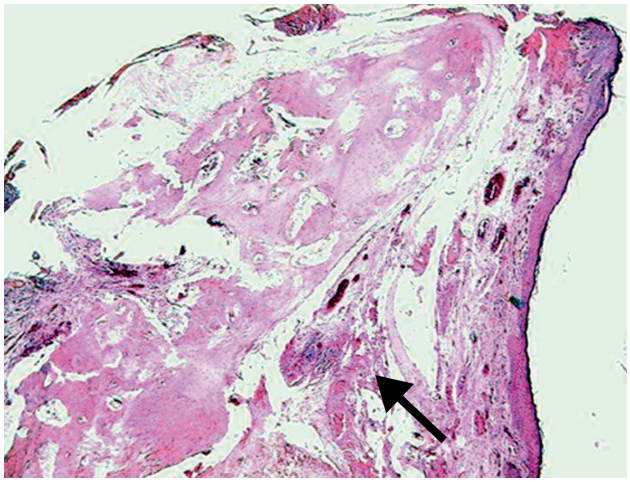

Fig. 2: Histological cross-section of incus with cholesteatoma (decalcification, haematoxylin and eosin staining, magnification 100×). The black arrowhead points to the cholesteatoma plug under thin bone lamella.

vasion of cholesteatoma into the vascular spaces or marrow spaces of the bone was observed.

Discussion

Autograft ossicles have been the choice for otologist for their biocompatibility, good hearing results and low cost. However, in patients with cholesteatoma the ossicles have been rejected because of a risk of residual disease. If the cholesteatoma is only superficial on the bone surface, mechanical cleaning of the ossicular surface should eliminate residual disease from the ossicles. Deeper invasion into the bone would exclude the possibility of mechanical cleaning of ossicles.

In this study, the residue of squamous cell epithelium was only superficially located. According to the literature, no sign of deeper invasion into the ossicle marrow was seen (2–10). One of our cases showed a plug of squamous cell epithelium underneath thin bone lamella. This finding explains the risk of residual cholesteatoma after ossicular stripping. If the surgeon eliminates the superficial soft tissue only by cold instruments (stripping), without drilling of the ossicular surface, the plug of squamous epithelium can persist underneath bone lamella. Ng et al. (6) found residual disease in 6 of 104 cleaned ossicles. Dornhoffer et al. (2) found residues in 7 of 11 specimens treated only by stripping without drilling. Vartiainen and Karjalainen (13) reported a low cholesteatoma recurrence rate of only 4%. The risk of residual disease is lowered by drilling of all ossicular surfaces under microscopic control and increased in cases of badly eroded ossicles (2, 6, 11, 12). Because badly eroded ossicles are deformed and flimsy, mechanical cleaning is technically more difficult and limited in efficacy. These severely eroded ossicles could be treated by autoclaving but badly deformed ossicles are usually not suitable for reconstruction of the ossicular chain (1, 11, 12).

We grouped the destruction of the malleus and incus into two grades. Our grading system is consistent with malleus and incus erosion classification (11, 12). Ossicle destruction grade I is mild erosion and ossicle is available for autograft ossiculoplasty. Ossicles with destruction grade II are badly eroded ossicles useless for ossiculoplasty.

Destruction grade II of incus is significantly more frequent compared to malleus. A badly eroded malleus was observed only in cases with badly eroded incus or completely destroyed incus. These findings could be explained by lower resistance of the incus against cholesteatoma. However, histological findings did not reveal important morphological differences between malleus and incus. In incus, large marrow spaces can persist this would not influence superficial erosion of the incus body. The persistence of large marrow spaces could be important for resorption of the long process of the incus in chronic otitis media. In the long process, there is only thin bone lamella protecting the bone marrow.

The second explanation for frequent destruction of the incus is the position of the cholesteatoma. Spread of cholesteatoma is consistent with the way the middle ear is ventilated. Preferential growth of cholesteatoma on the medial surface of the incus can explain its more frequent destruction as compared to the malleus.

Controversy exists as to whether cholesteatomas in childhood are more aggressive than cholesteatoma in adults. Multiple studies have shown that the rate of residual cholesteatoma is 2–3 times higher in children (14–16). Reasons for this difference are still quite unclear. Some have accented better-aerated mastoids in children in comparison with the usually sclerotic temporal bone in adults. A well-aerated mastoid provides an access of cholesteatoma to deeper aerated cells and more difficult elimination for surgeons. Current studies test levels of growth factors in cholesteatomas (17–19). Bujia et al. (18) haveproved a higher proliferation rate in pediatric cholesteatoma with increased levels of MIB-1; a nuclear antigen expressed by cells active in the cell cycle. De Carvalho Dornelles et al. (19) have demonstrated thicker epithelial matrices in pediatric cholesteatoma, higher levels of matrix metalloproteinases and exaggerated inflammatory profile. These findings suggest biologically more aggressive phenotype of pediatric cholesteatoma compared to adults. However, in our study, cholesteatoma in children was not found to be more aggressive to the middle ear ossicles. The ossicular destruction grade II in children was not significantly higher in comparison with adult cholesteatoma.

Conclusions

Cholesteatoma affects only superficial part of middle ear ossicles. A plug of squamous epithelium could spread underneath a thin bone lamella, but no deep invasion was observed. Autograft ossicles from cholesteatomatous ears should not necessarily be rejected for reconstruction of the ossicular chain. Regarding the histological finding, the authors suggest mechanical cleaning of the ossicle surface to eliminate residual disease.

Ethics committe approval

Ethics committee approval was received for this study from the ethics committee of University Hospital Hradec Králové (case number 200605 S07P).

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Conflict of interests

None declared.

Financial disclosure

None declared.

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