

Mechanical treatment and autoclaving of middle ear ossicles from cholesteatomatous ears

Research Article

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Abstract: Aim of the study: The authors present a prospective histomorphological study of middle ear ossicles, harvested from cholesteatomous ears, to evaluate for presence of residual cholesteatoma after mechanical treatment and short-time autoclaving. Materials and methods: Sixty ossicles were used as specimens in this study. The ossicles were grouped as follows: Group A - ossicles stripped and burred using a fine diamond burr under microscopic control. Group B - ossicles autoclaved for 4 min at 134°C after mechanical burring under microscopic control. Results: In group A, 30 ossicles were histologically examined after mechanical treatment. The residual disease was found in one badly eroded ossicle. In group B, 30 ossicles were histologically examined after mechanical treatment and autoclaving. That treatment eliminated cholesteatoma in all cases. The difference of results in these two treatment methods was not statistically significant. Conclusion: Authors recommend using mechanical cleaning in cases of mildly eroded ossicles. In cases of badly eroded ossicles authors recommend to either apply a combination of mechanical and autoclaving treatment. The study proved that 4 minutes of autoclaving at 134°C after mechanical cleaning provides safe bone autografts.

Keywords: Cholesteatoma • Autoclaving • Autograft ossiculoplasty

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1. Introduction

Autograft ossiculoplasty was first reported by Hall and Rytznar in 1957 [1]. The malleus and the incus have been utilized for tympanoplasty because of their advantages (in comparison with homologous materials or synthetic prosthesis) such as biocompatibility, price and long-term stability without risk of transmitting infection. However, their use in cholesteatoma ears has been limited by the possibility of harbouring within them residual cholesteatoma. There appears to be some evidence that the microscopic residuum of squamous cell epithelium in the ossicles could lead to reimplantation of the cholesteatoma [2-10]. The risk of cholesteatoma transmission using the autologous ossicles is widely accepted. But could we eliminate the residual cholesteatoma from the ossicles and prepare them for autologous ossiculoplasty without the risk of cholesteatoma transmission?

2. Materials and methods

Sixty middle ear ossicles used as specimens in this study. The ossicles were harvested from patients during surgery of chronic otitis media with cholesteatoma at Charles University Hospital in Hradec Kralove. Inclusion criteria were: chronic otitis media with cholesteatoma, confirmed attic cholesteatoma, clinical signs of cholesteatoma on the ossicle surface, surgery needs to have been carried out between 2006 and 2011.

The ossicles were grouped as follows:

Group A: 30 ossicles were stripped and burred using a fine diamond burr under microscopic control.

Group B: 30 ossicles were autoclaved for 4 min at 134°C after mechanical cleaning (stripped and burred ossicles using fine diamond burr under microscopic control).

All procedures were done by the same surgeon.

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The erosion of the malleus heads and body of the incudes were categorized as follows:

Grade I: size of the malleus head > 2 mm, size of the incus body with short process > 3 mm in a diameter.

Grade II: size of the malleus head < 2 mm, size of the incus body with short process < 3 mm in a diameter.

Middle ear ossicles were fixed in 10% formaldehyde and then decalcified by electrolysis in Sakura TDE 30 Decalcifier System. The tissue blocks were then serially sectioned for histopathological examination. The sections were stained with standard hematoxylin and eosin dyes and examined under light microscopy.

The subjects gave written informed consent.

The study was approved by the Ethics Committee.

The results were statistically evaluated by means of the Fisher Exact Test.

3. Results

In total 60 ossicles were examined from 36 patients for the study. There were 21 male and 15 female patients. Average of age was 36.9 years, median 37 years, age ranged from 5 to 73 years of age. Harvested ossicles included 32 malleus and 28 incudes. Serious erosion grade II was proven in 17 ossicles (malleus 6, incudes 11).

Serious erosion of the malleus was always associated with serious erosion or complete destruction of the incus. The erosion grade II or complete destruction of incus was found in 39% of all patients. The erosion grade II of malleus was found in 17% of all patients. The difference between incudes and malleus serious erosion is statistically significant ($p=0.0319$). According to the age of patient we proved erosion grade II in 4 cases from 13 children (31%) and in 10 from 23 adult patients (43%). The statistically significant difference was not proved ($p=0.501$).

Lymphocyte infiltration of the inner core of the ossicle was found in 3 cases.

In group A, 30 ossicles (malleus 16, incudes 14) were examined. Erosion grade II was detected in 5 ossicles. The surface of all 30 ossicles were stripped and burred under microscopic control. The mechanical treatment eliminated cholesteatoma in 29 ossicles. A residuum of squamous cell epithelium (Figure 1) was found in a severely eroded grade II ossicles in one case (3% of all cases). The confidence interval of that method in Fisher Exact Test (Lower 95% Confidence Limit – Upper 95% Confidence Limit) is 0.828 – 0.999.

In group B, 30 ossicles (15 malleolus, 15 incudes) were burred under microscopic control and autoclaved. In 8 ossicles the criteria of erosion grade II

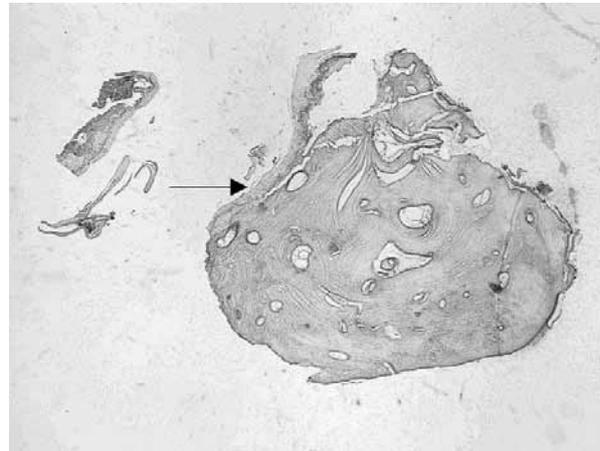


Figure 1. Histological cross-section of malleus head after mechanical treatment (decalcification, haematoxylin and eosin staining, magnification 100 x). The black arrowhead points to the residual squamous cell epithelium after treatment.

were met. The combination of mechanical and thermal treatment eliminated cholesteatoma in all cases. The ossicles had preserved their lamellar structure and no vital squamous cell epithelium was found. The inner cores of the ossicles were without lymphocyte infiltration (Figure 2). The confidence interval (Lower 95% Confidence Limit – Upper 95% Confidence Limit) is 0.884 – 1.0.

The difference of results in these two treatment methods was not statistically significant ($p=1.0$).

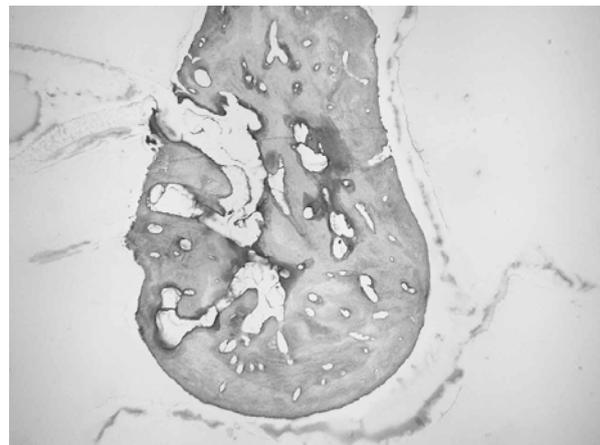


Figure 2. Histological cross-section of incus after mechanical treatment and autoclaving (decalcification, haematoxylin and eosin staining, magnification 100 x). The surface of the incus is without epithelium, the inner cores of the ossicle are without lymphocyte infiltration.

4. Discussion

The autologous malleus and incus have been used for tympanoplasty because of their biocompatibility, easy availability, good sound conduction and low cost of preparation [11-15]. Autograft ossicles maintain their contour, size, shape and physical integrity for long time, spanning at least 25 years [15-17]. Viability of ossicular autograft is not necessary from functional standpoint. Grafts that consist of nonviable bone appear to maintain their morphological structure and integrity and transmit sound as well as those consisting of viable bone [15-17]. However, their use in patients with cholesteatoma has been limited by the risk of harbouring residual disease. The changes in the middle ear ossicles in cholesteatomatous ears have been described in several histological studies [2-10]. However, the deeper invasion of cholesteatoma into the middle ear ossicles has never been published. The residual cholesteatoma has been found only superficially located in the presence of severe ossicular destruction.

The three main methods of treating cholesteatoma residue from ossicles have been published [2-4,6,18]. These are microscopic stripping, burring over the ossicular surface, and autoclaving. The risk of residual cholesteatoma after microscopic stripping is 6-64% [2,6]. This risk of residual disease could be reduced by burring of the ossicular surface [3,6] and increased in cases of badly eroded ossicles [2,6,18]. Available data of autoclaving of the middle ear ossicles have thus far been limited [3,4]. The autoclaving of the ossicles provides safe autografts but the autoclaving protocol 20 min at 134°C is time-consuming and proof of its effectivity is limited [3,4].

Our study compared two methods of treatment. The criteria for the methods of treatment were as follows: non time-consuming methods to operate in one-step surgery, low price and easy availability.

The first method was the mechanical cleaning of the ossicle by stripping and burring surface under microscopic control. In our study the residual disease was found in one case (3% of mechanical cleaning ossicles). It was only in a badly eroded ossicle (grade II). Because badly eroded ossicles are deformed and flimsy the mechanical cleaning is technically more difficult and lim-

ited in efficacy. These severely eroded and deformed ossicles are usually not suitable for reconstruction of the ossicular chain. The number of badly eroded ossicles is too small for statistical comparison between groups of ossicles grade I and grade II.

The second method was the autoclaving of the ossicle for 4 min at 134°C after mechanical cleaning. We chose our autoclaving protocol according to the studies of physical properties of the autoclaved bones. Several authors have published the influence of autoclaving on the mechanical properties of the bone and the effect on biological viability [19-21]. The duration of autoclaving has a greater negative influence on the mechanical properties of the bone than the temperature. Based on the available evidence our time-temperature protocol should decrease a negative effect on the physical properties of the bone with sufficient devitalisation effect to the epithelial cells. This protocol makes the bone treatment more convenient and less time consuming than the previously published procedure [3,4]. Our study proved that 4 minutes of autoclaving at 134°C after mechanical cleaning provides safe bone autografts.

5. Conclusion

This study suggests that autologous ossicles should not necessarily be rejected for reconstruction in cholesteatomatous ears. The surgeons should be able to remove the superficial disease if present. The risk of residual cholesteatoma could be decreased by stripping and burring of all ossicle's surface. The difference of results between mechanical treatment and combination of mechanical treatment and autoclaving was not statistically significant. Authors recommend using mechanical cleaning in cases of mildly eroded ossicles. In cases of badly eroded ossicles authors recommend to either apply a combination of mechanical and autoclaving treatment. The study also assessed the suitability of autoclaving at a shorter time in comparison with previous studies. The study proved that 4 minutes of autoclaving at 134°C after mechanical cleaning provides safe bone autografts.

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