Acquired Cholesteatoma of the Ear; Comparative Analysis of Histopathological Findings in Adults, Children and Recurrent Cases


ABSTRACT

Objective: To compare the histopathological characteristics of acquired cholesteatoma in adults, children and recurrent cases.

Methods: A retrospective analysis of 60 histopathological specimens for 60 patients aged 9 to 63 years who underwent otologic surgery for chronic otitis media with cholesteatoma was carried out at King Hussein Medical Centre between January 2006 till July 2010. Patients were divided into three groups as follows; group A patients aged > 16 years with no history of previous ear surgery, group B patients aged > 16 years and had history of previous otologic surgery for cholesteatoma and group C patients aged ≤ 16 years. Histopathological analysis was performed for specimens. Results for group A were compared with results of groups B & C separately.

Results: After histopathological analysis; atrophy was present in 26(84%) specimens in group A, 10 (71%) specimens in group B and 11(73%) specimens in group C. Twenty- seven (87%) specimens had acanthosis in group A, and (80%) in group C. Basal cell hyperplasia was present in 29 (94%) specimens in group A, 100% of group B, and 97% of group C. Epithelial cones were present in 20 (65%), 10 (71%), and 10 (67%) of our study groups respectively. Peri- matrix inflammation was present in 30(97%) of group A and 100% of both groups B and C. Results showed that there were no statistically significant differences between our study groups.

Conclusion: Although the sample size in this study was small but the statistical analysis showed that the histopathological characteristics of acquired cholesteatomas did not differ significantly between adults, children and recurrent cases. The characteristics of the peri-matrix should be analyzed more, especially in children to find if there is correlation with the behavior and aggressiveness of the disease.

Key words: Cholesteatoma, Histopathology, Recurrent cases.
temporal bone destruction due to mechanical pressure, enzymatically mediated bone resorption, and promotion of acute and chronic infections.(1,2) The growth of keratinocytes in the epidermis is regulated by a delicate balance between molecules that control cell survival and cell death. If this regulation is disturbed, epithelial cells may become pathological hyper-proliferative lesions, such as cholesteatoma.(3) Cholesteatoma has altered growth properties compared with the normal epidermal epithelium. It is characterized by an excessive growth of keratinocytes that leads to mucosal destruction.(4) The molecular and cellular processes resulting in the clinical hallmarks of cholesteatomas (i.e., migration, uncoordinated proliferation, altered differentiation, and aggressiveness) are not yet fully understood.(5) Annual incidence of cholesteatoma ranges around 3 in 100,000 in children and 9.2 in 100,000 in adults, and it is more predominant in male.(6,7) There are no medical therapies for cholesteatoma and current treatment is surgical resection. Recurrences are common and many individuals with cholesteatoma undergo multiple operations.(8,9) Histologically, the cholesteatomas consist of keratinized squamous stratified epithelium (matrix), with four layers identical to those of thin skin (basal, squamous, granulous, and stratum corneum), lying on a bed of connective tissue (peri-matrix).(10) According to Sudhoff H, the matrix is enclosed in a thin layer of connective tissue called the peri-matrix.(11) This is separated from adjacent bone by an inflammatory infiltrate which plays a decisive role in the potential spread of the cholesteatoma. (12,13) Cholesteatomas have hyperproliferating characteristics (14) with epithelial acanthosis, hyperplasia of the basal layer and the presence of epithelial cones in the matrix. This study was conducted to compare the histopathological characteristics of acquired cholesteatoma in adults, children and recurrent cases.

Methods
A retrospective analysis of 60 histopathological specimens for 60 patients aged 9 to 63 years who underwent otologic surgery for chronic otitis media with cholesteatoma was carried out. Ethical Committee Approval was obtained. This study was conducted at King Hussein Medical Centre (KHMC) for the period January 2006 till July 2010. Exclusion criteria included: absence of cholesteatoma matrix or perimatrix in the specimen and cases which were diagnosed as congenital cholesteatoma. Patients were divided into three groups as follows; group A patients aged > 16 years with no history of previous ear surgery, group B patients aged > 16 years and had history of previous otologic surgery for cholesteatoma and group C patients aged ≤ 16 years. Histopathological analysis was performed for specimens which were processed by routine histopathological techniques that included; 10% formaldehyde fixation, embedding in paraffin, cutting using the microtome, placing tissue sections on the slides and staining them with Hematoxylin and Eosin (H+E stain), and finally light microscopic examination. One serial number was used for each case. In our analysis five histopathological features were evaluated for these cases which were: atrophy which is defined as matrix with thickness of up to 4 keratinocyte layers, basal layer hyperplasia, acanthosis, epithelial cone formation, and perimatrix inflammation. Histopathological findings were assessed as absent or present and graded according to intensity as focal or predominantly atrophic for the first variant, and graded as mild, moderate and severe for acanthosis, basal layer hyperplasia and perimatrix inflammation. Results for group A were compared with results of groups B and C separately. Statistical analyses were performed with Statistical Package for Social Science - SPSS for Windows, using t-test and Fisher's exact tests when appropriate. All data are expressed as the mean ± standard deviation (SD). A value of P<0.05 was considered statistically significant.

Results
The patient's data was comparable between group A and B for age and gender (p < 0.05). Group A composed of 31 patients of whom 16(52%) were males and 15(48%) females; the mean age was 32.4 ± 17.2 years. Group B composed of 14 patients of whom 8(57%) were males and 6(43%) were females; the mean age was 31.3 ± 14.6 years. Group C composed of 15 patients of whom 7(47%) were males and 8(53%) were females; the mean age was 12.8 ± 2.3 years, (Table I).

After histopathological analysis; atrophy was present in 26(84%) specimens in group A, 10(71%) specimens in group B, and 11(73%) specimens in group C. The degree of atrophy was as follows; in group A, 10 (38%) was focal and 16 (62%) was predominant. In group B, 4 (40%) was focal and 6 (60%) was predominant. In group C, 4 (36%) was focal and 7 (64%) was predominant. Twenty- seven
Table I: Demographic data of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P value A versus B</th>
<th>P value A versus C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age Year</strong></td>
<td>32.4 ± 17.2</td>
<td>31.3 ± 14.6</td>
<td>12.8 ± 2.3</td>
<td>0.836</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Gender M:F</strong></td>
<td>16:15</td>
<td>8:6</td>
<td>7:8</td>
<td>0.492</td>
<td>0.500</td>
</tr>
<tr>
<td><strong>Total number</strong></td>
<td>31</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II: Histopathological findings in the study groups

<table>
<thead>
<tr>
<th>Histopathological feature</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P value A &amp; B</th>
<th>P value A &amp; C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy</td>
<td>Absent</td>
<td>5 (16%)</td>
<td>4 (29%)</td>
<td>4 (27%)</td>
<td>0.280</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>26 (84%)</td>
<td>10 (71%)</td>
<td>11 (73%)</td>
<td>0.312</td>
</tr>
<tr>
<td>Acanthosis</td>
<td>Absent</td>
<td>4 (13%)</td>
<td>1 (7%)</td>
<td>3 (20%)</td>
<td>0.461</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>27 (87%)</td>
<td>13 (93%)</td>
<td>12 (80%)</td>
<td>0.619</td>
</tr>
<tr>
<td>Epithelial cones</td>
<td>Absent</td>
<td>11 (35%)</td>
<td>4 (29%)</td>
<td>5 (33%)</td>
<td>0.461</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>20 (65%)</td>
<td>10 (71%)</td>
<td>10 (67%)</td>
<td>0.578</td>
</tr>
<tr>
<td>Basal layer</td>
<td>Absent</td>
<td>2 (6%)</td>
<td>0</td>
<td>1 (7%)</td>
<td>0.468</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>29 (94%)</td>
<td>14 (100%)</td>
<td>14 (93%)</td>
<td>0.701</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>Absent</td>
<td>1 (3%)</td>
<td>0</td>
<td>0</td>
<td>0.689</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>30 (97%)</td>
<td>14 (100%)</td>
<td>15 (100%)</td>
<td>0674</td>
</tr>
<tr>
<td>Perimatrix Inflammation</td>
<td>Absent</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Total No.</strong></td>
<td>31</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Cholesteatoma histopathology: keratinizing squamous epithelium with underlying inflamed perimatrix.

(87%) specimens have acanthosis in group A, of which 8 (30%) were mild, 10 (37%) were moderate and 9 (33%) were severe. It was absent in 4 (13%) specimens. In group B, acanthosis was present in 13 (93%) specimens and the degree was mild in 3 (23%), moderate in 6 (46%) and severe in 4 (31%) specimens, it was absent in 1 (7%) specimen. In group C, acanthosis was present in 12 (80%) and absent in 3 (20%) of the specimens, the degree was mild in 3 (25%), moderate in 6 (50%) and severe in 3 (25%) of the specimens. Basal cell hyperplasia was present 29 (94%) specimens in group A, 14 (48%) had mild degree, 11 (38%) had moderate and 4 (14%) had severe degree. In group B; all specimens had basal cell hyperplasia, 50% was mild, 5 (36%) had moderate and 2 (14%) had severe degree while in group C it was present in 14 (93%), absent in 1 (7%) and the degree was mild in 6 (43%), moderate in 5 (36%) and severe in 3 (21%) specimens. Epithelial cones were present in 20 (65%), 10 (71%) and 10 (67%) of our study groups respectively. Perimatrix inflammation was present in 30 (97%) of group A, 14 (47%) had mild degree, 9 (30%) had moderate and 7 (23%) had severe degree of inflammation. In group B, inflammation was present in all specimens examined (Fig. 1), 6 (43%) had mild degree, 5 (36%) had moderate degree and 3 (21%) had severe degree of inflammation. In group C, perimatrix inflammation was also found in all examined specimens, and the degree of inflammation was mild in 1 (7%) specimen, moderate in 3 (20%) and severe in 11 (73%) of the examined specimens. Table II represents the histopathological findings and statistical analysis of the study groups results showed that there were no statistically significant differences between the three study groups.

Discussion

Cholesteatomas were defined by Schuknecht as the accumulation of exfoliated keratin in the middle ear or any pneumatized area of the temporal bone,
deriving from a keratinized squamous epithelium. And it should be differentiated from congenital cholesteatoma which presents early in life as a white pearly mass behind an intact tympanic membrane.\textsuperscript{[16,17]} It may affect both children and adults, but there is controversy about its clinical behavior in the different age ranges in which it is manifested; Dornelles \textit{et al.}\textsuperscript{[18]} draw an analogy between the perimatrix and a “battlefield”, where there is a fight for the middle ear territory, between the cholesteatoma matrix itself and the adjacent tissues of the tympanic box. With the expansion of cholesteatoma, the inflammatory reaction would increase and therefore, it would produce more elements of the inflammatory cascade. TNF-\(\alpha\) is found in cholesteatomas, promoting bone resorption by different routes. It acts on osteoclast differentiation and maturation and also exposes the bone matrix.\textsuperscript{[19]} Ferlito \textit{et al.}\textsuperscript{[20]} described the perimatrix as the most peripheral portion of the cholesteatoma, comprising granulation tissue or inflammatory subepithelial connective tissue, with lymphocytes, histiocytes and neutrophils.\textsuperscript{[21]} Aural cholesteatomas vary in progression and aggressiveness; the presence of bacterial biofilms in some cholesteatomas may explain their activity.\textsuperscript{[22]}

By the histological analysis of cholesteatomas, Dornelles\textsuperscript{[23]} found an inverse correlation between the perimatrix size measured in micrometers and the age of patients at the day of surgery and that the degree of the perimatrix inflammation was strongly correlated with the perimatrix thickness. According to different authors,\textsuperscript{[24-26]} pediatric cholesteatoma is less expansive, which leads to lower incidence of complications. Conversely, others\textsuperscript{[27-31]} reported that acquired cholesteatoma in children should be presented in a more aggressive way and with more extensive growth. In our study we have not found any histopathological differences between cholesteatoma specimens of adults and children on one hand, and between specimens of adults who have first time surgery and those with revision surgeries. These results are comparable to what Dornelles and her colleagues\textsuperscript{[32]} and Leal Alves \textit{et al.}\textsuperscript{[13]} have found. We have noticed the higher intensity of perimatrix inflammatory response in children compared to adults but we failed to show statistically significant difference as Dornelles\textsuperscript{[23]} did. Quaranta \textit{et al.}\textsuperscript{[33]} proposed that the characteristics of the perimatrix may play an important role in the pathogenesis of the cholesteatoma, suggesting that these histo-

morphological characteristics would influence the recurrence and invasion of pediatric cholesteatomas. Dornelles and colleagues indicated that not only the perimatrix is more active in pediatric cholesteatomas, but also that the matrix would have a more active proliferation either current or past,\textsuperscript{[32]} and their findings corroborate the hypothesis from Bujia \textit{et al.}\textsuperscript{[29]} who suggested that pediatric cholesteatomas would present a more pronounced proliferative state. The aggressiveness of pediatric cholesteatomas may be also related to the histology of pediatric bone.

**Conclusion**

Although the sample size in this study was small but the statistical analysis showed that the histopathological characteristics of acquired cholesteatomas did not differ significantly between adults, children and recurrent cases. The characteristics of the perimatrix should be analyzed more, especially in children to find if there is correlation with the behavior and aggressiveness of the disease.

**References**


