Histopathological and immunohistochemical characteristics of acquired cholesteatoma in children and adults

Mohammed Bassiouny a, Nahed Badour b, Ahmed Omran a,*, Hani Osama a

a ENT Department, Alexandria Faculty of Medicine, Khartoum Square, Alexandria, Egypt
b Pathology Department, Alexandria Faculty of Medicine, Khartoum Square, Alexandria, Egypt

Received 6 February 2012; accepted 20 February 2012
Available online 17 March 2012

Abstract  Objective: To compare the histopathological structure and immunohistochemical characteristics of acquired cholesteatoma in children and adults.

Study design: Prospective clinical study in a tertiary care centers.

Material & methods: This study was conducted on 40 patients presenting with cholesteatomatous middle ear disease. Twenty patients were of a pediatric age group (<18 years) and the rest were adults (>18 years). Patients were admitted to either the ENT Department of Alexandria University Hospital or that of the Students’ Hospital. All cholesteatoma specimens were collected intraoperatively and preserved for histopathological examination and immunohistochemical technique (PCNA monoclonal antibody).

Results: Histopathological examination of the submitted specimens revealed that strips of stratified squamous epithelium with the underlying tissues were fibrous in adults while cellular inflammatory infiltrates were seen in children. The degree of fibrosis was significantly higher in the adult group. Immunohistochemical examination revealed significantly higher expression scores of...
1. Introduction

Cholesteatomas are cystlike, expansile lesions of the temporal bone lined by stratified squamous epithelium that contains desquamated keratin. They most frequently involve the middle ear and mastoid, but they may develop anywhere within the pneumatized portions of the temporal bone. They may be congenital or acquired. Acquired cholesteatoma has two varieties being primary (retraction pocket cholesteatoma) or secondary cholesteatoma. Acquired cholesteatoma of the middle ear is characterized by an intense inflammatory reaction that results in tissue and bone destruction. The cholesteatoma is characterized by the presence of a keratinized stratified squamous epithelium inside any air-filled area of the temporal bone. Histologically, it is composed of multilayered squamous epithelium (matrix) which is surrounded by a mesenchymatous granulation tissue (perimatrix). The epicenter of the osteolytic process is located in the contact zone between the matrix and perimatrix as well as signs for wound healing with the formation of granulation tissue and capillary multiplication that is predominant in the perimatrix.

2. Aim of the work

The aim of this work was to compare the histopathological structure and immunohistochemical characteristics of acquired cholesteatoma in children and adults.

3. Material and methods

This study was carried on 40 out of 312 patients (12.8%) presenting with cholesteatomatous middle ear disease from March 2009 to June 2010. They were admitted to the Department of Otorhinolaryngology in the Alexandria University Hospital and the Alexandria Students’ Hospital. Twenty patients were of the pediatric age group (<18 years) and the rest were of adults’ age group (>18 years).

Patient inclusion criteria in this study obeyed the following:

1. Diagnosis of cholesteatomatous chronic otitis media.

The degree of fibrosis was significantly higher in adult specimens indicating a reparative process and less invasiveness. PCNA expression was significantly higher in the matrix and perimatrix of pediatric cholesteatoma as correlated to its increased proliferation. Pediatric cholesteatoma was more aggressive and invasive than adult cholesteatoma.

Conclusion: The degree of fibrosis was significantly higher in adult specimens indicating a reparative process and less invasiveness. PCNA expression was significantly higher in the matrix and perimatrix of pediatric cholesteatoma as correlated to its increased proliferation. Pediatric cholesteatoma was more aggressive and invasive than adult cholesteatoma.

© 2012 Egyptian Society of Ear, Nose, Throat and Allied Sciences. Production and hosting by Elsevier B.V. All rights reserved.
Mann–Whitney test (a nonparametric test that compares two unpaired groups) was used as statistical test of significance to compare the degree of fibrosis and inflammation and PCNA expression between pediatric and adult specimens. Values of \( P \leq 0.05 \) were considered as statistically significant.

4. Results

All patients underwent surgery for an acquired middle ear cholesteatoma the operative specimens from 40 patients were collected. There were 20 adults, 7 males and 13 females, average age of 34.25 years (range 23–50 years); and 20 children, 8 boys and 12 girls, average age of 13.6 years (range 5–18 years).

The rates of conductive and mixed hearing loss were similar in both groups. The rate of severe degree of hearing loss was higher in the pediatric group (50% vs. 20%). Also there was one case of total hearing loss in the pediatric while there were no cases of total hearing loss in the adult group.

All patients underwent tympanomastoidectomy surgery, and disease extension, ossicular erosion, and degree of invasion were figured out. Specimens of cholesteatoma were collected. Seven specimens were excluded from the study (5 of the adult group and 2 of the pediatric group) because the tissue amount was insufficient for histopathological and immunohistochemical assessment (presented only corneal lamellae).

Regarding disease extension and ossicular invasion, in the pediatric group, there was a greater frequency of extension of the cholesteatoma into the whole mastoid cavity and middle ear (40% vs. 20%). The rate of extratemporal extension was higher in the pediatric group (10% vs. 0%). The rate of involvement of the whole ossicular chain was similar between both groups. However, the rate of invasion of the external auditory canal wall or tegmen was higher in the pediatric group (35% vs. 10%).

Histopathological microscopic examination of the submitted specimens revealed strips of stratified squamous epithelium showing no rete ridges (epidermal thickenings that extend downward between dermal papillae) (Fig. 1a). In some cases, only the epithelial element was detected with lamellar keratinized contents (Fig. 1b). Ghost squames (keratinized denucleated cells with an unstained, shadowy center where the nucleus has been) were frequently seen (Fig. 1c). Cellular dysplasias were not detected. There was normal nuclear to cytoplasm ratio. The underlying tissues were fibrous in adult cases (Fig. 2a) as compared to a more cellular inflammatory infiltrate (Fig. 2b) in cases of children. The inflammatory infiltrate was mainly composed of lymphocytes, histiocytes as well as plasma cells.

4.1. Histopathological examination

The degree of fibrosis was significantly higher in the adult group as compared to the pediatric group (Table 1). No differences

![Figure 1](image1.png) Section of a case of cholesteatoma showing (a) strip of stratified squamous epithelium (×200), (b) with a well developed lamellar keratin layer (×100) and (c) ghost cells (×400) (H & E stain).

![Figure 2](image2.png) The underling tissues were more fibrous in (a) adult cases (×100) and more cellular in (b) children’s cases (×200) (H & E stain).

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Adult (( n = 15 ))</th>
<th>Ped (( n = 18 ))</th>
<th>( Z (P) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inf.</td>
<td>Range 1.0–3.0</td>
<td>1.0–3.0</td>
<td>1.161 (0.246)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD 1.53 ± 0.74</td>
<td>1.83 ± 0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median 1.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Range 0.0–3.0</td>
<td>0.0–3.0</td>
<td>2.344 (0.019)*</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD 2.13 ± 1.06</td>
<td>1.22 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median 3.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Inf: inflammatory infiltrate. \( Z \): \( Z \) for Mann–Whitney test. * Statistically significant at \( P \leq 0.05 \).
were noted in the degree of inflammatory infiltrate between both groups. The mean scores of fibrosis and inflammatory changes in the perimatrix are listed in Table 1.

4.2. Immunohistochemical examination

Cholesteatomas in the pediatric group revealed significantly higher expression scores of the proliferation marker investigated.

4.1.1. In the matrix

The PCNA/LI of adult squamous epithelium (Fig. 3a) ranged between 1.0 and 48.0 with a mean of 17.75 ± 12.08. PCNA/LI of children’s cases (Fig. 3b) ranged between 8.0 and 67.0 with a mean of 32.82 ± 17.09. The differences between the PCNA/LI of the adult and children’s matrix were statistically significant (Table 2).

4.1.2. In the perimatrix

The PCNA/LI of adult inflammatory infiltrate (Fig. 4a) ranged between 0.0 and 64.0 with a mean of 14.77 ± 17.28. PCNA/LI of children’s cases (Fig. 4b) ranged between 0.0 and 76.0 with a mean of 24.22 ± 22.61. The differences between the PCNA/LI of the adult and children’s perimatrix were statistically significant (Table 2).

5. Clinico-pathological correlation

Pediatric cholesteatoma cases showed more extension and more invasion than adult cases, which may be correlated with the significantly increased expression of PCNA in both the matrix and perimatrix in the pediatric cases. In addition, the significantly increased fibrosis in the adult cases may be correlated with the less aggressive cholesteatoma in adults.

There was an impression that cases with higher proliferation scores (PCNA expression) showed more aggression (greater disease extension and greater degree of bone erosion). The expression of PCNA may be used as a prognostic marker for the aggressiveness and invasiveness of cholesteatoma.

6. Discussion

Acquired cholesteatoma of the middle ear characteristically presents with aggressive growth leading to the destruction of the ossicular chain and other surrounding bony structures. Histologically a multilayered squamous epithelium (matrix) is surrounded by a mesenchymatous granulation tissue (perimatrix). The epicenter of the osteolytic process is located in the contact zone between the matrix and perimatrix as well as signs for wound healing with the formation of granulation tissue and capillary multiplication being predominant in the perimatrix.

Table 2  Comparison between the two studied groups according to PCNA/LI of both matrix and perimatrix.

<table>
<thead>
<tr>
<th></th>
<th>Adult (n = 15)</th>
<th>Ped (n = 18)</th>
<th>Z (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prematrix</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.0–64.0</td>
<td>0.0–76.0</td>
<td>1.628 (0.014)*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>14.77 ± 17.28</td>
<td>24.22 ± 22.61</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>9.50</td>
<td>18.90</td>
<td></td>
</tr>
<tr>
<td><strong>Matrix</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.0–48.0</td>
<td>8.0–67.0</td>
<td>2.857 (0.004)*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>17.75 ± 12.08</td>
<td>32.82 ± 17.09</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>16.0</td>
<td>29.70</td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant at P ≤ 0.05.

![Figure 3](image1.png)  
(a) (b)

**Figure 3**  A case of cholesteatoma of (a) adults (×200) and (b) pediatric group (×400) showing nuclear positivity (red stained) in the surface epithelium (PCNA monoclonal antibody, peroxidase–antiperoxidase method).

![Figure 4](image2.png)  
(a) (b)

**Figure 4**  Section of two cases of cholesteatoma of an (a) adult (×200) and (b) pediatric (×400) cases. Note the differences in the PCNA positively staining nuclei (red stained) in both cases (PCNA monoclonal antibody).
Pediatric and adult cholesteatoma may behave in different ways. One might then hypothesize that pediatric and adult cholesteatomas might represent different spectrums of the same disease or even a different disease entirely. Clinically, it is not possible to make distinctions between the more and less aggressive cholesteatomas. The purpose of this study was to compare the histopathological structure and immunohistochemical characteristics of acquired cholesteatoma in children and adults, and consequently their impact on the clinical behavior.

The pediatric cholesteatoma in the present study demonstrated a higher incidence of active inflammation as evidenced by having more cellular perimatrix compared to the more fibrotic perimatrix in adult cholesteatoma.

In the present study, the examined histopathological specimens of pediatric and adult cholesteatoma revealed that pediatric perimatrix was more cellular in comparison to adult perimatrix. The degree of fibrosis was significantly higher in the adult specimens, which indicates that the adult cholesteatoma is more in reparative process and less invasive. No significant difference was noted in the degree of inflammatory infiltration between both groups, which indicates that the composition of inflammatory infiltrate is similar in both groups.

Cellular dysplasia, which is common in neoplastic lesions, was not detected. The cytogenetic findings combined with the histopathological characteristics (i.e. normal nuclear to cytoplasm ratio) which failed to demonstrate any signs of dysplasia, suggest that cellular dysregulation (neoplasia) is not the critical event in cholesteatoma genesis. These observations support those of many other investigators.

There are numerous studies reviewing the potential etiology of different behavior of pediatric cholesteatoma. Some investigators compared the histopathological structure of pediatric and adult cholesteatomas. They compared the histopathological components, perimatrix thickness and degree of inflammation between pediatric and adult cholesteatomas. Dornelles et al. showed no differences in the histopathological components of acquired cholesteatoma in adults and children. These results were not similar to the results in the present study. The significantly increased degree of fibrosis may be explained by more control of infection and inflammation in adult cases, better compliance and better preoperative preparation by medical treatment.

Welkoborsky et al. could not detect marked differences between adult and pediatric cholesteatoma on the cellular level. Dornelles et al. showed that the degree of inflammation of the perimatrix presented moderate to intense correlation with the perimatrix thickness. The perimatrix inflammation degree and size, measured in micrometers, presented an inverse correlation with patient’s age at the time of the surgery.

There were clues of a greater degree of inflammation in pediatric cholesteatoma. Thus it is suggested that the different clinical characteristics of pediatric cholesteatoma would be related to the amount of inflammation it causes. Dornelles et al. used matrix thickness as a parameter of comparison and they found no significant difference between both groups.

PCNA is a multifunctional protein with roles in DNA replication synthesis, DNA repair synthesis and recombination-driven DNA synthesis. It is a subunit of DNA polymerase, which has been associated with repair. It acts as sliding clamps needed for the activity of DNA polymerase and other enzymes from the battery used in DNA synthesis. For conclusion, PCNA has a triple function in the life and death of the cells. When not engaged in DNA replication, PCNA (most often under the control of p53) commits cells to cell cycle arrest and repair of DNA damage, or, when repair is not possible, absence or low levels of functional PCNA may drive cells into apoptosis.

PCNA has been used in cytological and histological material and has recently been reported to provide a simple method of recognizing proliferating cells in methacarn or alcohol fixed, wax embedded tissue, with results comparable with those achieved with flow cytometry. Therefore, it would also allow retrospective assessment of the proliferative rates in archival tissues due to the conservation of this marker in fixed and paraffin-embedded tissues.

PCNA expression was also tested in middle ear cholesteatoma. Olszewksa et al. used PCNA as a marker of proliferation in cholesteatoma. He found that its expression is significantly higher compared to normal postauricular skin. Shieh et al. also showed similar results. Other studies also confirmed these results.

These studies also showed that the expression of PCNA was present in the basal and suprabasal cell layers of cholesteatoma matrix, while it was expressed only in the basal layer of the skin.

In the present study, we used PCNA as proliferation marker to compare pediatric and adult specimens. PCNA expression was significantly higher in pediatric specimens compared to adult specimens. The expression was higher in both matrix and perimatrix. In the matrix, the mean expression of adult specimens was 17.75 ± 12.08, while the mean of pediatric specimens was 32.82 ± 17.09. In the perimatrix, the mean expression of adult specimens was 14.77 ± 17.28, while the mean of pediatric specimens was 24.22 ± 22.61. Increased expression of PCNA signifies increased proliferation, which may be correlated with increased invasiveness and destructive behavior of pediatric cholesteatomas.

In the present study, cholesteatoma with higher proliferation scores (higher PCNA expression) showed to be more aggressive (greater extension and more invasion). Therefore, PCNA expression may predict the severity of the disease. On the other hand, Hassmann-Poznanska et al. found no correlation between results of PCNA expression in cholesteatoma and clinical parameters.

The differences in the results of these various studies revealed that the increased proliferation is not the only explanation of the aggressive behavior of pediatric cholesteatoma.

Therefore we agree with Sade et al. that the clinically observed aggressive behavior of pediatric cholesteatoma may depend on other parameters, such as the disturbance of middle ear ventilation, the preformed paths for cholesteatoma extension in the middle ear, and/or reduced calcium salt content of the pediatric bone matrix.

### 7. Conclusion

The degree of fibrosis was significantly higher in adult specimens compared to pediatric specimens. This may indicate that adult cholesteatoma is more in reparative process and is less invasive. PCNA expression was significantly higher in the matrix and perimatrix of pediatric cholesteatoma compared to adult cholesteatoma.
adult cholesteatoma. This may be correlated with the increased proliferation of pediatric cholesteatoma. Pediatric cholesteatoma was more aggressive and more invasive than adult cholesteatoma.

References