ABSTRACT

OBJECTIVE: Paediatric cholesteatomas have been known with marked recidivism and aggressiveness. Hence, there are contradictory reports in this study, we aimed to test this hypothesis by histologically comparing paediatric and adult cholesteatomas.

MATERIAL and METHOD: Sixty-two adults, 15 children/adolescents who were operated between 2014-2016 made up the study and control groups.

For immunohistochemistry, neoplastic markers of K67 and proliferating cell nuclear antigen (PCNA); collagen type IV and fibronectin; inflammatory markers of keratinocyte growth factor (KGF); tumour necrosis alpha (TNF-α), interleukin-1 alpha (IL-1α) primary antibodies were used. The scores within (cholesteatoma/skin comparison) and between groups were statistically compared for each parameter.

RESULTS: In both groups, staining scores of all tested markers have been found significantly higher in the cholesteatoma matrices than the skin samples, except for collagen type IV. On the other hand, only lymphocytosis and PCNA expression differed at a closer statistical significance level between the groups, in favour of adults.

CONCLUSIONS: Acquired cholesteatoma is likely to be the same pathology in both paediatric and adult patients in many aspects of etiopathogenesis. However, inflammation appears to be playing more important role in the adult patients. The recidivism and aggressiveness of pediatric cholesteatomas are likely to be related to the proliferative effect of the growth factor and frequent inflammatory attacks.

Keywords: cholesteatoma, acquired, paediatric, pathogenesis, immunohistochemistry.

INTRODUCTION

Pediatric cholesteatomas have been known with rapid expansion and higher recurrence rate (1, 2). This observation was supported by the findings of Aslier et al (9), who diagnosed higher mitotic activity in the epithelium and stroma and an increased expression of TNF-α in the epithelium and stroma in comparison to healthy skin. Hence, adult cholesteatomas were considered indeed to show more aggressive biological behaviour (3). However, some other researchers have found no significant difference between pediatric and adult cholesteatomas in terms of proliferation rate and invasiveness, but intensity of inflammation (4, 5). Here, in this study, we aimed to test this hypothesis of different biological behaviour of pediatric and adult cholesteatomas using various parameters as histological, histometric, and histopathological parameters as to study neoplastic, invasive and inflammatory characteristics of the disease in both groups.

MATERIAL and METHOD

Between the years of 2014 and 2016, we used the cholesteatoma matrix and perimatrix granulomatous materials removed from 77 patients as study and control groups. In normal skin samples at the diameters of 1×0.3 mm harvested during the mastoidectomy from the same patients as control materials and sent for histopathology and immunohistochemistry (IHC) examination. Of these patients, 15 were pediatric patients (under the age of 18) between the ages of 5 to 18, whereas the remaining 62 adult patients’ ages varied 22 to 70. In order to IHC and H&E staining for microscopic examination, cholesteatoma tissue samples along with perimatrix granulomatous materials and external ear canal skin samples, harvested from each patient were fixed, embedded in paraffin, sectioned at 5 μ-thick sections for H&E staining and 4 μ-thick serial sections for IHC staining. The antibodies used for IHC examination marked were:

- Neoplastic markers of K67
- Proliferating cell nuclear antigen (PCNA)
- Invasive indicators of Collagen IV-VII and Fibronectine Inflammatory markers Keratinocyte growth factor (KGF)

Tumour necrosis alpha (TNF-α), Interleukin-1 alpha (IL-1α) primary antibodies were used.”

The staining level for PCNA is assessed around 45% in cholesteatoma, acquired, paediatric, pathogenesis, immunohistochemistry. However, inflammation appears to be playing more important role in the adult patients. The recidivism and aggressiveness of pediatric cholesteatomas are likely to be related to the proliferative effect of the growth factor and frequent inflammatory attacks.

**RESULTS:**

In both groups, staining scores for K67 (%), PCNA (%)(Fig 1 a, b), KGF, TNF-α, IL-1α; vascular structure and lymphocyte counts (in H&E stained slides) have been found significantly higher in the study materials (cholesteatoma matrix and perimatrix) than the control materials (p>0.001). However, for the collagen type IV the staining score was lower in cholesteatomas than the control materials (p>0.001), and non-different for collagen type VII, for which the p score was <0.05. When the scores of pediatric and adult cholesteatomas are compared, in none of the tested IHC and H&E staining parameters no statistically significant difference (when the p value is taken as >0.05) was found. However, for the collagen type VII the p value was 0.0084 the level of significance were PCNA and the number of lymphocytes and as P=0.0738 and 0.2167 respectively in favour of adult cholesteatomas.

**DISCUSSION**

Cholesteatoma recidivism has been found significantly higher in children than adults, although hearing loss and compression rates are comparable (2). This more aggressive and recurring nature of pediatric cholesteatoma than its adult counterpart has been attributed to the following factors: a. children undergo more frequent acute otitis media attacks and this would play a role in cholesteatoma etiopathogenesis. b. Better pneumatized mastoids in children allow rapid and more sizeable expansion of cholesteatomas and c. the activity of growth hormone that stimulates epithelial proliferation in pediatric age group. As the manifestations of cholesteatoma have been demonstrated that the keratin proliferation rate is higher in children as well as metalloproteinase expression that is responsible for the destructive nature of the disease (3, 5, 6). However, Aslier et al. found that epithelial thickness and Ki-67 expression is higher in adults contrary to the above conclusions (7). Our findings are also in line with the results of the study. Welkoborsky et al also found that both pediatric and adult cholesteatomas have similar features at cellular level, and suggested that aggressive behavior of pediatric cholesteatoma is probably due to the intensity of inflammation and poor middle ear ventilation (Welkoborsky et al 4). Dornelles et al attributed to the aggressiveness of pediatric cholesteatomas to the intensity of inflammation and its putative indicator of thickness of perimatrix layers (8). However, our results contradicted this finding as the inflammatory finding of inflammation and its putative indicator of thickness of perimatrix layers (8). However, our results contradicted this finding as the inflammatory finding of lymphocytosis was more pronounced in adult cholesteatomas, although not at statistically significant level. On the other hand, again statistically insignificant, yet considerably higher expression of proliferative marker of PCNA in adults needs to be clarified through further studies. Likewise, the results of several studies failed to confirm the theory that cholesteatoma is a low-grade neoplasia despite exhibiting high mitotic activity (9).

**CONCLUSION**

The relative aggressiveness and recidivism of pediatric cholesteatoma seemed to be related to other factors such as higher mitotic capacity of epithelium and more frequent acute exacerbations of chronic middle ear inflammations, rather than biological differences between pediatric and pediatric cholesteatomas.

**REFERENCES**


**Table 1**

<table>
<thead>
<tr>
<th>Staining parameter</th>
<th>Pediatric Cholesteatoma</th>
<th>Adult Cholesteatoma</th>
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<tbody>
<tr>
<td>PCNA</td>
<td>0.75 ± 0.23</td>
<td>0.47 ± 0.15</td>
</tr>
<tr>
<td>Ki-67</td>
<td>0.56 ± 0.12</td>
<td>0.38 ± 0.09</td>
</tr>
<tr>
<td>KGF</td>
<td>0.62 ± 0.20</td>
<td>0.45 ± 0.15</td>
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<tr>
<td>Collagen type IV</td>
<td>0.70 ± 0.25</td>
<td>0.58 ± 0.18</td>
</tr>
<tr>
<td>Collagen type VII</td>
<td>0.55 ± 0.18</td>
<td>0.43 ± 0.14</td>
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**Figure 1**

(a) Positively stained inflammatory cells in the cholesteatoma perimatrix (×200).

(b) Positively stained proliferating cell nuclear antigen (PCNA) in cholesteatoma perimatrix (×200).

**Figure 2**

(a) Staining for Ki-67 and PCNA in cholesteatoma perimatrix (×200).

(b) Staining for Ki-67 and PCNA in cholesteatoma perimatrix (×200).

**Figure 3**

(a) Staining for Ki-67 and PCNA in cholesteatoma perimatrix (×200).

(b) Staining for Ki-67 and PCNA in cholesteatoma perimatrix (×200).