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Analysis of histopathological aspects and bone destruction characteristics in acquired middle ear cholesteatoma of pediatric and adult patients

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ABSTRACT

Objective: The aim of this study was to investigate the proliferative and apoptotic activity of middle ear cholesteatoma in pediatric and adult patients, in addition to comparing its histopathological aspects and the severity of advanced bone destruction.
Materials and methods: Medical records of 223 patients treated for chronic otitis media with cholesteatoma at the Otolaryngology Department of Dokuz Eylul University between January 1992 and December 2013 were retrospectively evaluated. Sixty-one patients subjected to tympanomastoidectomy due to middle ear cholesteatoma, with sufficient specimens for histopathological examination, were included in the study. Sections of archived tissues in paraffin blocks were subjected to new histopathological examinations. The proliferative and apoptotic activities of cholesteatoma were determined by immunohistochemical staining for epithelial thickness (ET), and Ki-67 and caspase-3 expression. A novel scoring system, the Bone Erosion Score (BES), was developed to estimate the severity of bone destruction. The Austin–Kartush classification score (AKCS) was also calculated.
Results: ET and Ki-67 expression was higher in adult patients than in the pediatric patients ($p = 0.009$ and 0.01, respectively); however, caspase-3 immunopositivity did not show any significant intergroup differences ($p = 0.106$). The differences in AKCS and BES between pediatric and adult patients were not statistically significant. According to the correlation analysis, a significant positive correlation was observed between AKCS and BES ($p = 0.001$), and between ET and Ki-67 expression (when histopathological data were compared) ($p = 0.001$).
Conclusion: The proliferative activity of cholesteatoma was higher in adult patients. Therefore, these findings do not support the theory that the aggressive clinical course of cholesteatoma in pediatric patients is correlated with its histopathological characteristics.

1. Introduction

Cholesteatoma is characterized by a destructive and expanding growth of keratinizing squamous epithelium in the temporal bone [1]. The clinical profile of cholesteatoma includes aggressive growth leading to the destruction of the ossicular chain and other surrounding bony structures. The hyperproliferative nature of the epithelium could potentially contribute to its highly aggressive biological behavior [2–5]. Pediatric patients have been shown to frequently demonstrate a more aggressive growth pattern and a higher recurrence rate compared with adults [6,7]. Although multiple studies have attempted to investigate this feature by comparing histopathological findings in pediatric and adult patients, the molecular mechanism of cholesteatoma remains controversial. Uncoordinated cell proliferation may be an important pathophysiological mechanism in the development of cholesteatoma [2–5].

The biological activity of cholesteatoma has been evaluated by immunohistochemical examinations of cholesteatoma specimens in several previous studies [3–5]. It has been hypothesized that the aggressive pattern of pediatric cholesteatoma may be explained by

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its histopathological composition, which affects cellular proliferation, apoptosis, and the cell cycle. Ki-67 and caspase-3 were selected to evaluate the proliferative and apoptotic activity of cholesteatoma in the present study.

The Ki-67 protein is a nuclear protein associated with cellular proliferation, and can be detected during all active phases of the cell cycle (G_1, S, G_2, and M) [3]. Therefore, it is a suitable marker for the determination of the proliferative activity of a cell population. Caspase-3 is a member of the cysteine-aspartic acid protease family, and plays a dominant role in the execution-phase of cell apoptosis [8,9]. In addition to staining, epithelial thickness (ET) was calculated as an additional proliferative indicator in the present study.

The destructive clinical activity of cholesteatoma was evaluated by the Austin–Kartush Classification Score (AKCS) [10,11] and a novel scoring system called the Bone Erosion Score (BES), which assesses bone defects in the middle ear ossicles, tegmen, facial canal, and the lateral semicircular canal.

Therefore, the primary aim of this study was to investigate the proliferative and apoptotic activity of middle ear cholesteatoma in pediatric and adult patients. In addition, the proliferative and apoptotic activity of the cholesteatoma epithelium was compared with the severity of advanced bone destruction.

2. Material and methods

2.1. Patients and samples

A total of 223 patients who underwent tympanomastoidectomy due to acquired chronic otitis media with cholesteatoma at the Otolaryngology Department of Dokuz Eylul University, İzmir, Turkey, between January 1992 and December 2013, were retrospectively identified by a systematic search of medical records. Patients treated for primary surgeries were selected and patients whose first surgeries were performed for recurrent or residual cholesteatoma were excluded. All of the available microscopic slides of these patients were carefully reviewed. At the end of histopathological overview, the patients whose surgical materials lacked enough epithelial tissue were also excluded from the study. Finally sixty-one patients, whose cholesteatoma specimens comprised adequate epithelial tissue, were included in this study. Patients under the age of 18 comprised the pediatric group and those aged 18 years and older comprised the adult group.

2.2. Histopathology and immunohistochemistry

Histopathological examinations were performed on formalin-fixed and paraffin-embedded cholesteatoma specimens obtained during surgery. New sections were prepared and stained with hematoxylin and eosin (H&E). The most representative microscopic slides (containing adequate specimens) were selected for immunohistochemical staining.

The ET of each H&E-stained section was measured between the basal membrane and the surface of the epithelium in 20 different fields, and the average length was calculated for each patient. For immunohistochemical Ki-67 staining, the sections were initially incubated with cell conditioning 1 (CC1) solution at 95 °C for 52 min, and then incubated with Ki-67 (clone MIB1 [ready-to-use]; Dako, Glostrup, Denmark) antibody solution for 32 min. For caspase-3 staining, sections were incubated with CC1 solution at 95 °C for 64 min, followed by incubation with a caspase-3 antibody (E8, SC-7272; Santa Cruz Biotechnology, Dallas, TX, USA) solution for 32 min. The entire staining process was performed using the BenchMark ULTRA (Ventana Medical Systems, Tucson, AZ, USA) fully automated immunohistochemistry staining platform, supplemented with an Ultraview universal DAB detection kit (Ventana Medical Systems). The results of the Ki-67 staining were recorded as the percentage of 1000 epithelial cells displaying positive nuclear staining. Caspase-3 was evaluated semi-quantitatively; staining of weak intensity was classified as a negative result, whereas strong-intensity staining was accepted as a positive result. All sections were analyzed using a Nikon Eclipse Ci light microscope (Nikon Corporation, Tokyo, Japan), and digitalized using the Nikon DS-Fi2 (Nikon Corporation).

2.3. Severity of bone destruction

Bone defects in the middle ear ossicles, tegmen, facial canal, and the otic capsule were determined based on the intraoperative findings recorded in the patients' surgical records. Based on these parameters, two classification strategies were utilized to investigate the destructive process in the temporal bone and the ossicular chain. The first method was the AKC, composed by Austin in 1972, and Kartush modified it in 1994 as a part of the "Middle Ear Risk Index" [10,11]. Although the AKCS describes seven groups, only the ossicle head fixation (group E) and stapes fixation (group F) groups are discussed in this study. The AKCS was determined for each patient, and groups A, B, C, and D were scored as 1, 2, 3, and 4, respectively. The mean scores of the pediatric and adult groups were assessed.

However, the tegmen, facial canal, and the otic capsule must be evaluated in addition to the ossicles, because of their significant role in cholesteatoma complications. Therefore, a novel scoring system, the "BES", was developed in this study to specify the intensity of bone destruction in the temporal bone and ossicular chain. Based on the surgical observations, the samples were scored as either '1' for recognizable ossicle in the presence of partial destruction or '2' for indistinguished ossicle in the presence of complete destruction. This scoring system examined all ossicles, with the exception of the footplate of the stapes, which was regarded as a part of the otic capsule. The tegmen tympani, facial canal, and the otic capsule were assessed separately. These temporal bone sections were also evaluated by BES due to their significant role in the development of complications related to cholesteatoma. However, partial defects in these areas could not be comprehensively distinguished. Therefore, the presence of full-thickness bone erosion was scored as 2 in the BES system. Overall, all scorings were done one by one and added together to generate the total erosion score (Table 1).

3. Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences v.15.0 (IBM, Armonk, NY, USA). Mean values and standard deviations were calculated. The mean values of measurable variables were compared within the groups by the Mann–Whitney *U* test. Categorical variables were analyzed by the

Table 1
The definition and marking system of 'Bone Erosion Score'.

	Intensity of the bone destruction		
Ossicular temporal bone structure	Normal	Partial	Complete
Malleus	0	1	2
Incus	0	1	2
Stapes (footplate excluded)	0	1	2
	Bony defect		
Non-ossicular temporal bone structure	None		Present
Tegmen tympani	0		2
Facial canal	0		2
Otic capsule (footplate included)	0		2