ACQUIRED CHOLESTEATOMA IN CHILDREN AND ADULTS - A CLINICO-PATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF ITS CHARACTERISTICS

P. Benjamin Kumar¹, K. Srinivas²

¹Assistant Professor, Department of ENT, Government ENT Hospital, Koti, Attached to Osmania Medical College, Hyderabad.

²Assistant Professor, Department of ENT, Government ENT Hospital, Koti, Attached to Osmania Medical College, Hyderabad.

ABSTRACT

BACKGROUND

Cholesteatoma (CH) is a bag-like cystic structure developing in the middle ear cleft, either following a retraction pocket or epithelial migration or metaplasia. It forms in Chronic Suppurative Otitis Media (CSOM) of attic-antral type. Though the pathogenesis of CH is same in adults and children, its clinical presentation is more aggressive in the latter. Many factors play their role in producing osteolytic changes in the walls of middle ear cleft. One among them is an evidence of cellular histochemical reactions initiated by enzymes, bacterial proteins and toxins liberated following lysis of the organism.

The aim of this study is to compare the histopathological structure and immuno-histochemical characteristics of acquired cholesteatoma in children and adults.

Study Design - Prospective clinical study in tertiary care centre.

MATERIALS AND METHODS

86 patients presenting with clinical diagnosis of CSOM with cholesteatoma are included; 35 patients belonged to the age group below 18 years and the remaining 51 were above 18 years. Patients were examined under microscope, audiometry and per-operative assessment of the disease was done. Tissue specimens were collected per-operatively and preserved for histopathological examination and immuno-histochemical technique (PCNA monoclonal antibody).

RESULTS

HPE study showed layers of stratified squamous epithelium with the underlying tissue fibrosis in adults when compared to children, which was significant statistically with a p value 0.015. High infiltrations of inflammatory cells were seen in both adults and children. Immuno-histochemical examination revealed higher expression scores of proliferation markers significant statistically with p values of 0.0054 (p value taken as significant < 0.05).

CONCLUSION

The adult CHs showed reparative process in the form of fibrosis, but equal inflammatory infiltration as found in Children. The higher PCNA values in matrix and peri-matrix epithelium of children may be correlated to more aggressive and invasive nature of the cholesteatomas.

KEYWORDS

Cholesteatoma, PCNA, Inflammation, Matrix, Perimatrix, Children, Adult.

HOW TO CITE THIS ARTICLE: Kumar PB, Srinivas K. Acquired cholesteatoma in children and adults - a clinico-pathological and immunohistochemical study of its characteristics. J. Evolution Med. Dent. Sci. 2017;6(3):163-166, DOI: 10.14260/Jemds/2017/40

BACKGROUND

Collection of desquamated keratin in the center with multiple layers of squamous epithelium (Matrix) surrounded by mesenchymatous granulation tissue (Perimatrix) growing in the middle ear cleft in acquired type of CSOM may progress to produce osteolysis in its vicinity.¹ CH may also develop in other parts of temporal bone. The perimatrix of the CH shows the intense cellular activity converting the dormant osteoclasts to produce osteolysis.^{2,3,4} The center of activity of CH is the contact zone between matrix and perimatrix.

Presence of budding capillaries, immature fibroblasts, acute inflammatory infiltrates in perimatrix.^{5,6,7,8} CHs have the potential to destroy the skull bones in its vicinity including ossicles and bony labyrinth.^{6,9} Cholesteatomas occurring in children are usually observed to have an aggressive spread and frequent growth pattern and higher recurrence rate than those observed in adults.¹⁰ The present study is an attempt to compare the different clinical and histopathological changes of CH in adult and paediatric patients to understand the reason for this increase, which was poorly understood.

MATERIALS AND METHODS

The present study was conducted at Government ENT Hospital attached to Osmania Medical College, Hyderabad. The study period was between May 2012 and April 2014; 86 patients were included in the study out of 11326 patients attending with CSOM during this period. Group A consisted of 35 patients aged below 18 years and Group B consisted of the remaining 51 patients aged above 18 years.

Financial or Other, Competing Interest: None.

Submission 04-12-2016, Peer Review 28-12-2016,

Acceptance 04-01-2017, Published 09-01-2017.

Corresponding Author:

Dr. P. Benjamin Kumar,

Assistant Professor,

Department of ENT,

Government ENT Hospital, Koti, Hyderabad,

Attached to Osmania Medical College, Hyderabad.

E-mail: pbenzrk@yahoo.co.in

DOI: 10.14260/Jemds/2017/40

Inclusion Criteria

Patients diagnosed to have CH on microscopic examination of the ear. Patients showing per-operatively with presence of distinctive perimatrix and matrix to be collected as tissue specimens.

Exclusion Criteria

Patients with congenital CH. Patients with limited CH. All the patients were explained of the procedure and a written consent obtained. Institutional Ethical Clearance Certificate was obtained. Demographic data of the sample of patients was elicited. A thorough history of otorrhoea and loss of hearing was obtained. Microscopic examination of the ears in the OPD was done to select appropriate patients for the study. Audiological evaluation and surgical profile was done. All the patients admitted for CH surgery were subjected to CT scan of the temporal bones to evaluate pre-operatively the status of dural plate, sinus plate, facial nerve and labyrinthine fistulae. Patients were subjected to modified radical mastoidectomy (Canal Wall Down - CWD) and CHs were removed. During surgery the peri-matrix and matrix were identified and specimens collected into 10% Formalin solution to fix the tissue. The status of the ossicular chain was graded according to standard classification. All the specimens were prepared and reported by a single pathologist who was blinded about the nature of the tissue. The tissue sections were studied under Haematoxylin and Eosin stain (HPE), Masson Trichrome for the assessment of the degree of fibrosis and immuno-histochemical technique for PCNA (Proliferating Cell Nuclear Antigen) monoclonal antibody [PC10 (anti-PCNA)]. The immuno-histochemistry staining was performed using the peroxidase-antiperoxidase method. To compare the results of two groups of patients Easy Fisher test exact test was used as statistical test of significance to compare the degree of fibrosis and inflammation and PCNA expression. A p value below 0.05 was taken as statistically significant.

RESULTS

In the Group A patients (35), there were 27 males and 8 females. Among the Group B (51), 36 were males and 15 were females. All patients underwent surgery for an acquired middle ear cholesteatoma. The operative specimens from all patients were collected. Group A patient's mean age was 7.2 ± 2.4 (Age range: 5 to 14 years). Group B patient's mean age was 28.62 ± 6.2 .

(Age range: 15 to 43 years), (Table 1).

Groups	Male - 27	Female - 08	Age Group	Number
Group A	07	04	05 - 09 Yrs.	11
	16	08	10 - 14 Yrs.	24
Group B	Male - 36	Female - 15		
	09	05	15-24	16
	10	07	25-34	19
	17	03	35-44	20

Table 1. Showing the Age and Gender Incidence (n = 86)

In Group A, attic perforation was observed in 12 (34.28%), Posterior marginal perforation in 7 (20%) and large central perforation in 16 (45.71%) patients. In Group B, patient's attic perforation was observed in 24 (47.05%), posterior marginal perforation in 18 (35.29%) and large

central perforation in 17 (33.33%) patients. History of loss of hearing was observed in both the groups, but better expressed by the patients of Group B. The type of hearing loss was mixed in nature on audiometry in both the groups. The Pure Tone Averages (PTA) of Air Conduction (AC) hearing were 40 dB to 55 dB in 13 patients (37.14%) and 56 dB to 65 dB in 22 patients (62.85) of Group A. The hearing loss among higher frequencies; 3000 KHZ to 6000 KHZ in Group A was 50 dB to 75 dB. The PTA of AC hearing was 30 dB to 45 dB in 29 patients (56.86%) and 46 dB to 50 dB in 22 patients (43.13%) of Group B; the hearing loss among higher frequencies; 3000 KHZ to 6000 KHZ in Group B was 45 dB to 50 dB. Total Hearing Loss (THL) was observed in 3 patients of Group A and none in the Group B. In Group A patients the extent of CH was limited to attic in 4 patients (11.42%), limited to attic and antrum in 7 patients (20%) and involvement of middle ear cleft and entire mastoid air cells up to the tip in 24 (68.57%). In Group B patients the extent of CH was limited to attic in 18 patients (35.29%), limited to attic and antrum in 13 patients (25.49%) and involvement of middle ear cleft and entire mastoid air cells up to the tip in 20 (39.21%). The difference in terms of percentage of destruction of Group A patients was higher (88.57%) compared to 64.70% in Group B. Ossicular erosion was showing higher grades of involvement in Group A (80%) compared to Group B (60.78%) (Table 2).

Observation	Group A		Group B	
	No	%	No	%
Central Perforation	16	45.71	24	47.05
Attic Perforation	12	34.28	18	35.29
Marginal Perforation	07	20	17	33.33
PTA - AC				
40 dB - 55 dB	13	37.14	29	56.86
56 dB - 65 dB	22	62.85	22	43.13
Extent of Disease				
Limited to Attic	4	11.42	18	35.29
Attic + Antrum	7	20	13	25.49
Attic + Antrum + Tip of Mastoid	24	68.57	20	39.21
Ossicular Status				
M+, I+, S+	07	20	20	39.21
M+, I-, S+	12	34.28	17	33.33
M+, I-, S-	16	45.71	14	27.45

Table 2. Showing the Comparison between Group A & B in Various Clinical, Audiometry and Operative Findings (n = 86)

Groups	Group A - 35		Group B - 51		P Value
Observation	No	%	No	%	
Fibrous Tissue	08	22.85	36	70.58	0.015
Inflammatory Cellular Infiltration	30	85.71	45	88.23	0.0054
PCNA Expression	32	91.42	16	31.37	0.0054

Table 3. Showing the HPE, Cellular Infiltrates and PCNA Expression in Both Groups (n = 86)

HPE showed no rete ridges, (epidermal thickenings that extend downward between dermal papillae). There were no cellular dysplasias. There was normal nuclear to cytoplasm ratio. The underlying tissues were showing fibrous proliferation in Group B specimens which was significantly higher when compared to Group A patients, but there was no

difference in cellular inflammatory infiltrates. Lymphocyte, plasma cells and histiocytes infiltration was predominant in the inflammatory infiltrates. Immuno-histochemical examination of the specimens from Group A showed a higher expression of proliferation markers in the matrix and perimatrix (91.42%) when compared to Group B patients (31.37%), which was statistically significant with p values below 0.05 (Table 3). Finally, the clinico-pathological correlation of Group A patients showed aggressive invasion of CH, ossicular destruction, moderate mixed HL compared to Group B patients. The HPE of the CH matrix and peri-matrix showed more fibrosis in Group B compared to Group A. The cellular infiltrates were equal in both the groups. The immuno-histochemical markers were presenting with a higher expression in Group A compared to Group B.

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.¹¹ One can then hypothesise that paediatric and adult cholesteatomas may be representing different spectrums of the same disease or even a different disease entity. As clinically it is difficult to make distinctions between the more and less aggressive cholesteatomas, a study such as this would help to compare the HPE of tissue and quantify the cellular activity in them with the help of immuno-histochemical study was to compare the histopathological structure and cellular proliferates by immuno-histochemical methods. The paediatric CHs of children in the present study showed a higher incidence of active inflammation as observed by presenting more cellular perimatrix compared to the more fibrotic perimatrix of adult CHs. The degree of fibrosis under the perimatrix was significantly higher in adult CH specimens. It may suggest that the adult CHs are more of a wound healing process and so not invasive. At the same time, no difference in the degree of inflammatory infiltration noted between both the groups. Hence, one can assume that the composition of these infiltrates is same in both the groups. No cellular dysplasias or neoplastic changes were observed. These observations are supported by many other authors.^{12,13,2} Few authors compared only the HPE of CHs of children and adults. They based their studies on histopathological components, perimatrix thickness and degree of inflammation between paediatric and adult CHs. Dornelles¹⁴ et al showed no differences in the histopathological components of acquired CH in adults and children in contrast to the present study. Hypothetically, the presence of more fibrosis in adult CH suggests better control of infection and inflammation giving them better compliance in treatment. In a similar study by Welkoborsky et al,¹² authors did not have definite differences between adult and paediatric CHs on the cellular level. Dornelles et al^{14,15} showed that the degree of inflammation of the perimatrix presented moderate to intense correlation with the perimatrix thickness. De Dornelles et al¹⁶ used

matrix thickness as a parameter of comparison and they found no significant difference between both groups. PCNA is a protein and sub-unit of DNA polymerase with multiple functions such as DNA replication synthesis, DNA repair synthesis and recombination driven DNA synthesis; a triple function in the life and death of the cells. Absence or low levels of functional PCNA may drive cells into apoptosis.¹⁷ PCNA expression was also tested in middle ear cholesteatoma. Olszewska et al¹⁸ used PCNA as a marker of proliferation in CH. He found that its expression is significantly higher compared to normal post-auricular skin. Shieh et al¹⁹ also showed similar results. In the present study, PCNA was used as a proliferation marker to compare paediatric and adult specimens. PCNA expression was significantly higher in paediatric specimens compared to adult specimens. Increased expression of PCNA signifies increased proliferation, which may be correlated with increased invasiveness and destructive behaviour of paediatric CHs. In the present study, paediatric CH showed higher markers for PCNA and clinically was more aggressive (Greater extension and more invasions compared to adults). But Hassmann-Poznanska et al²⁰ found no correlation between results of PCNA expression in cholesteatoma and clinical parameters. The contradicting result of different studies with the present study concludes that the increased proliferation alone cannot be the explanation of the aggressive behaviour of paediatric CHs. Therefore, one has to concur with Sade et al²¹ that the clinically observed aggressive behaviour of paediatric CH may depend on other parameters such as the disturbance of middle ear ventilation, the preformed paths for CH extension in the middle ear and/or reduced calcium salt content of the paediatric bone matrix.

CONCLUSION

Adult cholesteatomas with marked fibrosis in the perimatrix compared to children represent a reparative process at a higher grade and lower invasiveness. Presence of cellular infiltrates equally in both children and adult CHs suggest an ongoing chronic inflammatory process following infection. Higher expression of PCNA in matrix and perimatrix of children correlate well with their clinical and audiological findings compared to less aggressive adult CHs.

REFERENCES

- [1] Telian SA, Schmalbach CE. Chronic otitis media. In: Snow JB, Ballenger JJ, eds. Ballenger's otorhinolaryngology head and neck surgery. 16th edn. Hamilton, Ontario, BC Decker Inc 2003:261-93.
- [2] Albino AP, Kimmelman CP, Parisier SC. Cholesteatoma: a molecular and cellular puzzle. *Am J Otol* 1998;19(1):7-19.
- [3] Aberg B, Westin T, Tjellstrom A, et al. Clinical characteristics of cholesteatoma. *Am J Otolaryngol* 1991;12(5):254-8.
- [4] Ferlito A, Devaney KO, Rinaldo A, et al. Clinicopathological consultation. Ear cholesteatoma versus cholesterol granuloma. *Ann Otol Rhinol Laryngol* 1997;106(1):79-85.
- [5] Lim DJ, Saunders WH. Acquired cholesteatoma: light and electron microscopic observations. *Ann Otol Rhinol Laryngol* 1972;81(1):1-11.

- [6] Kurihara A, Toshima M, Yuasa R, et al. Bone destruction mechanisms in chronic otitis media with cholesteatoma: specific production by cholesteatoma tissue in culture of bone-resorbing activity attributable to interleukin-1 alpha. *Ann Otol Rhinol Laryngol* 1991;100(12):989-98.
- [7] Wright CG, Meyerhoff WL. Pathology of otitis media. *Ann Otol Rhinol Laryngol Suppl* 1994;163:24-6.
- [8] Kuczkowski J, Kobierska-Gulida G, Izyska-Swieszewska E, et al. Molecular control of bone resorption in chronic otitis media with cholesteatoma. *Otolaryngol Pol* 2010;64(4):219-24.
- [9] Orisek BS, Chole RA. Pressures exerted by experimental cholesteatomas. *Arch Otolaryngol Head Neck Surg* 1987;113(4):386-91.
- [10] Shohet JA, de Jong AL. The management of pediatric cholesteatoma. *Otolaryngol Clin North Am* 2002;35(4):841-51.
- [11] Sudhoff H, Bujia J, Borkowshi G, et al. Basement membrane in middle ear cholesteatoma. Immunohistochemical and ultrastructural observations. *Ann Otol Rhinol Laryngol* 1996;105(10):804-10.
- [12] Welkoborsky HJ, Jacob RS, Hinni ML. Comparative analysis of the epithelium stroma interaction of acquired middle ear cholesteatoma in children and adults. *Eur Arch Otorhinolaryngol* 2007;264(8):841-8.
- [13] Mallet Y, Nouwen J, Lecomte-Houcke M, et al. Aggressiveness and quantification of epithelial proliferation of middle ear cholesteatoma by MIB1. *Laryngoscope* 2003;113(2):328-31.
- [14] Dornelles C, da Costa SS, Meurer L, et al. Correlation of cholesteatomas perimatrix thickness with patients age. *Braz J Otorhinolaryngol* 2005;71(6):792-7.
- [15] Dornelles C, Meurer L, da Costa SS, et al. Histologic description of acquired cholesteatomas: comparison between children and adults. *Braz J Otorhinolaryngol* 2006;72(5):641-8.
- [16] Dornelles Cde C, da Costa SS, Meurer L, et al. Comparison of acquired cholesteatoma between pediatric and adult patients. *Eur Arch Otorhinolaryngol* 2009;266(10):1553-61.
- [17] Paunesku T, Mittal S, Protic M, et al. Proliferating cell nuclear antigen (PCNA): ringmaster of the genome. *Int J Radiat Biol* 2001;77(10):1007-21.
- [18] Olszewska E, Chodynicky S, Chyczewski L, et al. Some markers of proliferative activity in cholesteatoma epithelium in adults. *Med Sci Monit* 2006;12(8):CR337-40.
- [19] Shieh TJ, Ho KY, Kuo WR, et al. Evaluation of proliferative activity in middle ear cholesteatoma using proliferating cell nuclear antigen. *Kaohsiung J Med Sci* 1999;15(8):468-74.
- [20] Hassmann-Poznanska E, Skotnicka B, Dzieciol J. Markers of epidermal proliferation in middle ear cholesteatoma. *Otolaryngol Pol* 2003;57(4):505-11.
- [21] Sade J, Fuchs C. A comparison of mastoid pneumatization in adults and children with cholesteatoma. *Eur Arch Otorhinolaryngol* 1994;251(4):191-5.