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ORIGINAL ARTICLE

Morphologic changes of middle ear mucosa in chronic otitis media with or without cholesteatoma

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OBJECTIVE: To investigate histopathologic differences between chronic otitis media (COM) with cholesteatoma and COM without cholesteatoma.

MATERİALS AND METHODS: This retrospective study is an analysis of 74 middle ear biopsies from the promontory near the round window taken at first operation for COM performed. Thirty ears had COM with cholesteatoma. The other 44 ears had COM without cholesteatoma. Materials were stained by Hematoxylin-eosin and Toluidine blue. Density of gland and secretory cell, epithelial thickness, number of ciliated cell, infiltration and migration of chronic inflammatory cells (lymphocyte and plasma cell) and grade of vascular dilatation and proliferation between the patients with or without cholesteatoma were compared. The analysis of quantitative parameters was performed using Pearson χ^2 test.

RESULTS: Infiltration and migration of lymphocyte and plasma cells, and grade of vascular dilatation and proliferation were significantly greater in ears without cholesteatoma than those with cholesteatoma.

CONCLUSİONS: These findings indicate that distinct physiopathologic mechanisms may play role in development of COM in terms of presence of cholesteatoma. Cholesteatoma may lead to destruction of vascular structures and chronic inflammatory cells by means of its pressure effect, numerous enzyme and cytokines.

The lining of the middle ear is a modified respiratory epithelium although ciliated and non-ciliated cells accumulate on different part of the middle ear cleft. Morphological aspects of the normal and pathological ear have been studied by several authors giving a more precise definition to the pathogenesis and clinical behavior of middle ear cholesteatoma. Naturally, the epithelium reacts to the inflammatory irritation whether there is cholesteatoma or not. However, inflammatory changes of the middle ear mucosa demonstrate different patterns under the chronic influence of the middle ear disease. Glandular hyperplasia, giant cells, acantosis, keratosis and squamous metaplasia, lymphocytic cell infiltration are the frequent microscopic changes which have seen in the middle ear mucosa [1, 2, 3].

The severity of middle ear tissue changes can affect post-operative hearing results. Ojala and Sorri found that mean post-operative air-bone gap was significantly better in ears with mild histopathological changes [4]. There have been some studies on histopathology of the middle ear mucosa. However, the morphologic changes of the middle ear mucosa and chronic otitis media with or without cholesteatoma have not been studied and compared analytically. 74 middle ear mucosa specimens, which were taken during surgery of chronic middle ear disease with or without cholesteatoma, were investigated and morphologic findings were compared.

MATERIALS AND METHODS

The study is a prospective analysis of 74 middle ear biopsies taken at operation for chronic suppurative otitis media with or without cholesteatoma. Subjects were 16 female and 58 male with the age between 4 and 66. Mean age was 26.5±11.9 years. Tissue biopsies were obtained from the promontory near the round window during surgery of the patients by using cup biting forceps. Biopsies were prefixed in 10% formaldehyde and immediately transported to the histology laboratory for microscopic analysis under the light microscopy. Materials were stained by Hematoxylin-eosin and Toluidine Blue. Intra-operative findings related to the type and extend of disease were documented. Thirty ears had chronic otitis media with cholesteatoma . All cholesteatomas were primary acquired cholesteatoma that was extended tympanic cavity and mastoid antrum. Cholesteatomas contacted almost all tympanic mucosa excepting hypotympanic mucosa. The other forty-four ears had chronic otitis media without cholesteatoma. Forty-one ears without cholesteatoma had also polypoid formation. Eighteen patients underwent myringoplasty operation only. Twenty-six patients (2 cholesteatomatous) received intact canal wall technique. Thirty patients underwent canal wall down mastoidectomy. Only three cases were revised. Cholesteatoma has been found in 2 of revision operation.

Density of the glands was determined according to the scanning as the average number of glands in each scanned area. Gland count was classified as follows; Slight: no gland or less than 1, Moderate: 1-2 gland, Increased: more than 2. In the middle ear, the thickness of the epithelium normally consists of one or two-layered cuboidal epithelium. The epithelium was scored according to the pathological examination as follows. 0: a layer of pseudo-stratified epithelium, 1: a thin layer of pseudo-stratified epithelium, 2: moderate degree thickness of pseudo-stratified epithelium, 3: severe degree thickness of pseudo-stratified epithelium (hyperplastic). The number of ciliated cells was determined according to the scanning as the average number of ciliated cells in each scanned area. Cell count was classified as follows. Slight: no ciliated cell or less than 1, Moderate: 1-2 ciliated cells, Increased: more than 2 ciliated cells. To measure the severity of infiltration and migration of lymphocytic cells, first, the total number of cells was calculated. Then, it was graded as the number of cells per square millimeter to classify the severity of cell infiltration. After completion of counting of all specimens, the cell

count was rated in 3 groups as slight, moderate and severe. Vascular dilatation and proliferation was scored according to the pathological examination and it was graded as slight, moderate and severe. The analysis of quantitative parameters was performed using Pearson χ^2 test. (SPSS 11.5 program, SPSS Inc.,Chicago, IL., USA). A value of $p<0.05$ was considered statistically significant.

RESULTS

All the biopsies taken from draining ears presented intensive inflammatory reaction with proliferation of numerous new capillaries accompanying inflammatory cell infiltration. Results of histopathologic assessments and their comparisons between the groups are seen in Table 1, 2, 3, 4 and 5. Mononuclear inflammatory cell infiltration was one of the characteristic findings in the middle ear mucosa regardless of the presence of cholesteatoma. However, infiltration and migration of lymphocytic cell was significantly more in COM without cholesteatoma group than COM with cholesteatoma group ($p<0.01$). There was infiltration and migration of mononuclear inflammatory cells in a rate of 60% in COM with cholesteatoma group whereas it occurred in a rate of 97.7% in COM without cholesteatoma group (Slight, moderate and increased). Grade of vascular dilatation and glandular proliferation was likewise considerable ($p=0.001$). Glandular metaplasia was in close relation with the dysfunction of the Eustachian tube which tells more about the true nature of the chronic secretory and polypoid otitis media of long duration. Slight, moderate and severe vascular dilatation and

Table-I: Density of gland on histopathologic examination

Group	Slight	Moderate	Increased	P	χ^2
COM with cholesteatoma	80%	10%	10%	0.239	2.863
COM without cholesteatoma	68.2%	25%	6.8%		

Slight: no gland or less than 1, **Moderate:** 1-2 gland, **Increased:** more than 2.

Table-2: Score of epithelial thickness on histopathologic examination

	Score					
Group	0	1	2	3	P	χ^2
COM with cholesteatoma	50%	30%	16.7%	3.3%	0.926	0.468
COM without cholesteatoma	43.2%	36.4%	15.9%	4.5%		

0: a layer of pseudo-stratified epithelium, **1:** a thin layer of pseudo-stratified epithelium, **2:** moderate degree thickness of pseudo-stratified epithelium, **3:** severe degree thickness of pseudo-stratified epithelium (hyperplastic).

Table-3: Density of ciliated cell on histopathologic examination

Group	Slight	Moderate	Increased	P	χ^2
COM with cholesteatoma	80%	10%	10%	0.961	0.079
COM without cholesteatoma	77.3%	11.4%	11.4%		

Slight: no ciliated cell or less than 1, **Moderate:** 1-2 ciliated cells, **Increased:** more than 2 ciliated cells.

Table-4: Infiltration and migration of mononuclear (lymphocytes and plasma cells) inflammatory cells on histopathologic examinatio.

Group	No	Slight	Moderate	Increased	P	χ^2
COM with cholesteatoma	40%	16.7%	26.7%	16.7%		
COM without cholesteatoma	2.3%	6.8%	36.4%	54.5%	<0.01*	23.101

*: Statistically significant . It was graded as the number of cells per square millimeter to classify the severity of cell infiltration.

Table-5: Grade of vascular dilatation and proliferation on histopathologic examination.

Group	No	Slight	Moderate	Increased	P	χ^2
COM with cholesteatoma	46.7%	30%	10%	13.3%	0.001*	16.134
COM without cholesteatoma	11.4%	27.3%	9.1%	52.3%		

*: Statistically significant. It was scored according to the pathological examination.

proliferation, totally, were found in a rate of 88.6% in COM without cholesteatoma group versus 53.3% in COM with cholesteatoma group. There was no island of mucosal metaplasia in the middle ear in any of the specimens. In the overwhelming majority of cases the epithelium was thick and subepithelial tissue was edematous. A considerable amount of hyalinization was observed regardless of presence of cholesteatoma. Histopathologic stained samples can be seen in Figure-1,-2,-3,-4 and -5.

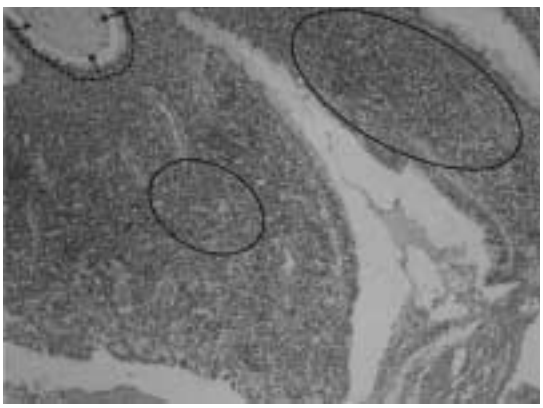

Figure-1: Lymphocyte-rich stroma (ovals) and glandular formation in patients with obvious eustachian tube dysfunction: abundant mucus (arrows) at the lumen of the secretory glands (dotted line) lined with columnar epithelium and increased goblet cells are seen. (HEx100).

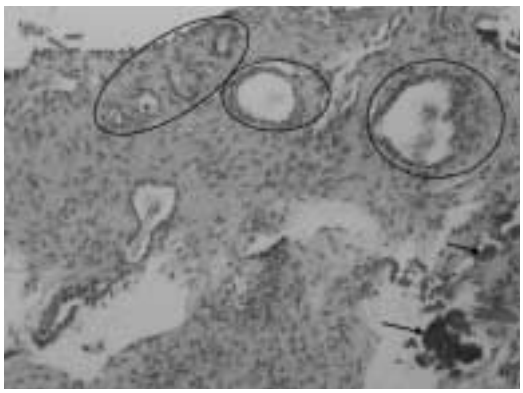

Figure-2: Proliferated mucous glandular formations (ovals) and calcifications (arrows) are seen in edematous and connective tissue. (HEx200).

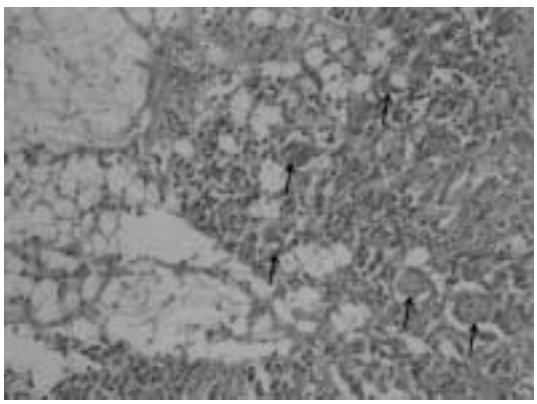

Figure-3: Keratin plaques and foreign body giant cell (arrows) reaction against keratin are seen. (HEx200).

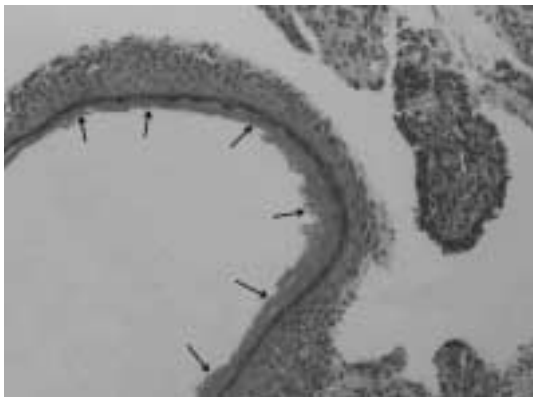

Figure-4: Typical cholesteatoma with thick keratinization (arrows). (HEx200).

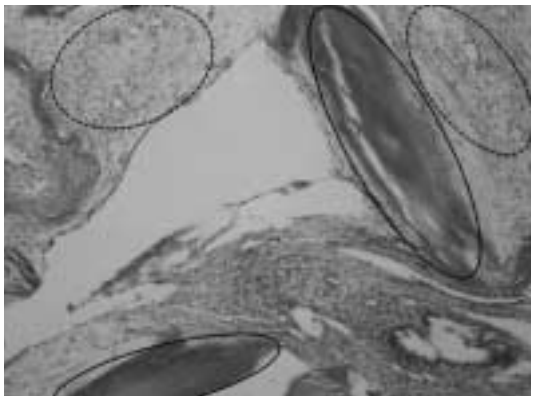

Figure-5: In patients with long-term ear drum perforation, hyalinization and sclerosing the collagenous tissue and the calsification deposits (ovals) are the prominent scene. Fibrosis and infiltration of chronic inflammatory cells (dotted ovals) at the connective tissue is evident. (HEx100).

DISCUSSION

Chronic middle ear disease is characterized by the appearance of several histopathological changes. Middle ear epithelium under chronic infection conditions shows hypertrophy, hyperplasia, secretory proliferation and squamous metaplasia. Increasing of secretory elements, e.g. goblet cells and secreting glands may give rise to different clinic and pathologic stages of the disease [2, 3, 5]. As the infection subsides the cells turn to normal. However, in the presence of persistent chronic infection, the ability of the epithelium to restore itself fails and degeneration of the cells with the expansion of differentiated epithelium and the hyperplasia of the basal cells proceeds until the triggering stimulus is eliminated [6]. Regression to the original form from the previous recurrent inflammatory condition does not take place and the mucous membrane remains ready to react even to minimal irritation [7]. Thus the severity, duration and the extent of the disease plays major role for the deterioration of the middle ear mucosa. Eventually, mucosal transformation comes to an irreversible point and is so extensive that the surgery is unavoidable. Mucosal response to chronic irritation is briefly indicative of the severity of the disease. If the biopsies at the time of surgery mirror the terminal stage of the epithelial damage, it is good to compare the chronic otitis media with or without cholesteatoma if there is any difference in worsening pattern.

Lymphocytes and plasma cells are predominant cells but polymorphonuclear leucocytes are also seen and macrophages are present in varying numbers [8]. Palva and Taskinen [3] in a histopathologic study of determination of lymphocyte subset in patients with chronic otitis media have found that 8 of 13 (61.5%) specimens with cholesteatoma had very thin non-inflamed membrane with infiltration of a few lymphoid cells whereas the other 5 specimens consisted of thick epithelium with large sub-epithelial inflammatory infiltration by large numbers of lymphocytic cells. They also found that infected cholesteatoma membrane had low T-helper/inducer:T-suppressor/cytotoxic ratio. Inflammatory cell type of the mucosa is likely to be altered by the chronic infection [3]. Van der Beek reported that tubal obstruction of the rat resulted with stratification, hyperplasia or transformation of the epithelium and the increase in the number of secretory elements [9]. Tos et al have demonstrated a sequence of histopathologic changes following one another and have found vasodilatation, edema, distention of the epithelial cells due to fluid uptake and increase in the secretory

elements like goblet cells and mucous glands which occur without infection, after long-term tubal occlusion in cats [10]. Transformation of the original epithelium into mucus-producing cells was found experimentally when the middle ear infection developed. Mechanical obstruction of the Eustachian tube due to edema or cellular infiltration causes an effusion of the fluid into the middle ear cavity with impairment of mucociliary function initially and then promotes a series of pathological reactions [11]. Prolongation of the problem seems to be one of the causes for the severity of the mucosal dysfunction.

Atef et al investigated ciliary count in COM patients using electron microscopy and image analysis, and compared the ciliary count between mucosal and squamous types [12]. It was found that cases of cholesteatoma were associated with much lower ciliary area values and thus much more ciliary destruction than the mucosal type (p=0.002; highly significant). They also found that in cases of COM, there was a statistically valid and quantitatively proved ciliary destruction when compared with normal controls. They supposed that ciliary destruction is result of a direct effect of bacterial toxins, excessive mucus secretion or inflammatory products. Regarding the mucus secretion as one of the causes of ciliary destruction, we have found in our study that the difference in the gland density was not statistically significant between the groups (p=0.261). Other different immunologic and physiopathologic mechanisms may play role rather than glandular hyperplasia and epithelial transformation since we have found similar rates of submucosal gland, epithelial thickness and ciliated cells in both groups. Vascular dilatation and mononuclear inflammatory cell infiltration were significantly higher in patients without cholesteatoma in the presented study. Meyerhoff et al have found more frequent inflammation and granulation in chronic otitis media without cholesteatoma after histopathological examination of pathologic temporal bones [13]. On the other hand, cholesteatoma of the middle ear excretes numerous enzymes or various cytokines which may affect cell infiltration [14-16]. Yetiser et al have demonstrated that the level of TNF-alpha and interleukins are not the same in patients with or without cholesteatoma [17]. In the presented study, inflammation was much more severe in patients without cholesteatoma.

Conclusively, the characteristic finding of the chronic middle ear inflammation was found to be lymphocyte infiltration, thickened connective tissue layer (lamina propria) with edema and proliferated mucosal epithelium in this study. None of the biopsy materials in this study demonstrated solitary metaplastic islands even in the presence of cholesteatoma. There was a less chance to find normal mucosa in patients with middle ear disease with or without cholesteatoma lasting longer period. The duration of the disease seems to be proportional with the deterioration of the middle ear mucosa. However, inflammation was much more severe in patients without cholesteatoma in the presented study. Hyalinization and sclerosing scar tissue was prominent in non-draining ears where as hyperplasia and abundant secretory proliferation was the major view in draining ears. There seems to be a breaking point for the self-healing process in such that the middle ear mucosa no longer exists although the chronic disease subsides. It is interesting to note that why infllamation in COM with cholesteatoma was less than COM without cholesteatoma in our study. One may consider that several reasons might play a role in that. One of the reasons might be the fact that cholesteatoma was likely to lead to destruction of vascular structures that decreased surge of the inflammatory cells to the field. Second reason might be that survival of chronic inflammatory cells was compromised due to pressure effect of cholesteatoma, enzymes and cytokines. COM with cholesteatoma especially primarily acquired cholesteatoma starts without inflammatory cells at the beginning. Inflammation in those case may be secondarily involved in. In contrary, COM without cholesteatoma is primarily inflammatory process in origin.

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