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ORIGINAL ARTICLE

Chronic suppurative otitis media with cholesteatoma and chronic otitis media with granulation investigated by scanning electron microscope based on an analysis of 140 patients

Authors' Contribution:
A–Study Design
B–Data Collection
C–Statistical Analysis
D–Data Interpretation
E–Manuscript Preparation
F–Literature Search
G–Funds Collection

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SUMMARY:

Introduction: Chronic purulent cholesteatoma of the middle ear, as well as to a lesser extent chronic granulomatous otitis media, lead to destruction of bone structures within the middle ear space. The above process is controlled by the OPG/RANKL/RANK system.

Aim: The aim of the study is a comparative analysis of surgical material obtained from patients with diagnosed chronic cholesteatoma and chronic otitis media with inflammatory granulation in the assessment of the Scanning Electron Microscope (SEM).

Material and methods: An analysis of 140 patients operated on due to chronic otitis media was performed. Forty patients who had been diagnosed with chronic cholesteatoma of the middle ear and chronic granulomatous otitis media were selected for a detailed analysis in the SEM. The final study under SEM included 20 patients.

Results: The regular structure of cholesteatoma depicted in the SEM concerned 5 patients. In the remaining 7 patients, the system was irregular and even chaotic. The lack of regularity can also be observed in the case of granulation tissue, which in the SEM image presented itself as an irregular tissue mass without detectable regularities.

Conclusions: (1) The regular pattern of the cholesteatoma matrix cells observed in some patients with chronic cholesteatoma of the middle ear reduces the molecular permeability of inflammatory cytokines, concurrently limiting the destructive activity on bone structures; (2) the presence of inflammatory granulation tissue in the middle ear is accompanied by an influx of leukocytes: neutrophils and lymphocytes, which are the source of pro-inflammatory cytokines, the growth of which activates the processes leading to the damage of bone tissue and the development of inflammation; (3) no specimen of acquired cholesteatoma revealed the presence of commensal organisms from Demodex species on the surface of the exfoliated human epithelium.

KEYWORDS: bone defects, chronic otitis media, inflammatory granulation, scanning electron microscope, structure of cholesteatoma

INTRODUCTION

Chronic otitis media is characterized by a defect of the tympanic membrane, periodic leakage from the ear and various degrees of conductive or mixed hearing impairment. Depending on the observed changes in the lining of the middle ear, several types of chronic otitis media are distinguished: chronic simple otitis media, chronic otitis media with inflammatory granulation, chronic cholesteatoma of the middle ear and chronic otitis media in the course of specific diseases [1, 2].

Cholesteatoma is a focal lesion, formed by the accumulation of exfoliated multilayered squamous keratinized epithelium as well as keratin and cholesterol deposits along with numerous inflammatory mediators. The incidence of cholesteatoma is 3–6 per 100,000 people. It can be classified as congenital and acquired. The expansively growing epidermal masses of cholesteatoma are the site of secondary bacterial infections, which are often resistant to conservative treatment.

Chronic otitis media with granulation is characterized by mucopurulent leakage from the ear. Bleeding, vivid red granulation tissue is formed on the basis of inflammatory changes. This condition requires differentiation from the proliferative process.

Chronic purulent cholesteatoma of the middle ear, as well as to a lesser extent chronic otitis media with granulation, lead to destruction of bone structures within the middle ear space. Bone resorption in cholesteatoma and inflammatory granulation of the middle ear is controlled by the OPG/RANKL/RANK system, regulated by proinflammatory cytokines and interactions between bone tissue cells and activated immune cells [3–6].

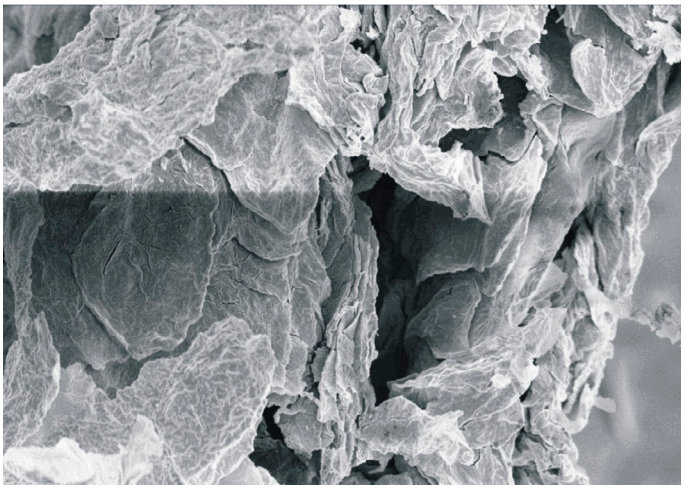

Fig. 1. Matrix. Magnification x1000. Focal corneocyte desquamation in the matrix. Squamous keratinocytes of the matrix, showing unordered stacking pattern and irregular microplicae on the surface. The Laboratory of Scanning Microscopy.

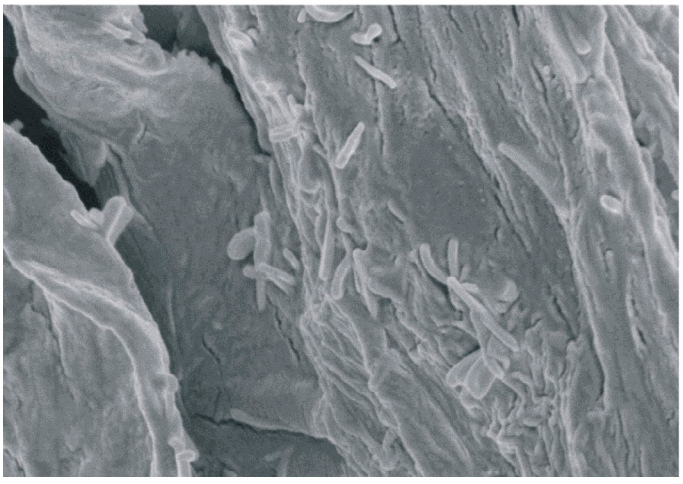

Fig. 2. Perimatrix/Matrix, Magnification x4000. Singular bacterial colonies on the border perimatrix/matrix. The Laboratory of Scanning Microscopy.

METHODS

The aim of the study is a comparative analysis of surgical material obtained from patients with diagnosed chronic cholesteatoma and chronic otitis media with inflammatory granulation in the assessment of the Scanning Electron Microscope (SEM).

An analysis of 140 patients operated on due to chronic otitis media was performed between 2017–2018. Forty patients were selected for detailed analysis in the scanning electron microscope, all of whom had been diagnosed with chronic cholesteatoma of the middle ear and chronic otitis media with inflammatory granulation. Due to damages of the analyzed material making evaluation under the electron microscope impossible, which arose during the preparation of the preparations and after the initial assessment of the usefulness of samples for evaluation in SEM, the study included 20 patients:

- N1 chronic cholesteatoma of the middle ear – 12 patients,
- N2 chronic otitis media with inflammatory granulation – 8 patients.

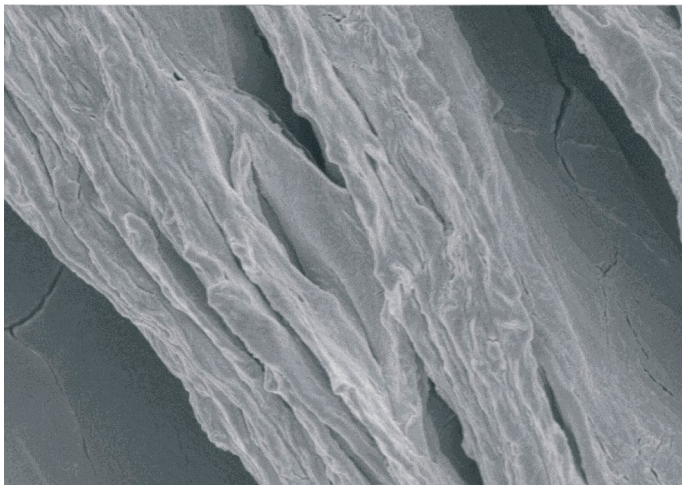

Fig. 3. Squamous keratinocytes of the matrix, regular microplicae on the Surface, magnification x2000. The Laboratory of Scanning Microscopy.

All patients underwent histopathological examination, the result of which determined the final diagnosis.

The removed operating material was prepared for analysis under a Scanning Microscope, by fixing in 4% formalin, and dehydration in a range of ethyl alcohols with concentrations: 35%, 50%, 75%, 96%, 99.9% (absolute alcohol). The preparations were air dried, and subsequently glued on the tiles by using an adhesive (Electrodag 915 Silver Paint, TAAB). The material prepared in this way was sprayed with a thin layer of gold using a JEOL JEE-4x (Vacuum Evaporator) device. Then, the examination was performed using the JEOL JSM35CF scanning microscope at the Scanning Microscopy Laboratory.

The outcomes were analyzed statistically with the value of $p < 0.05$ considered significant for all tests.

The bioethics committee issued approval no. 1072.6120.2.2017 for this study on 18 May 2017. Informed consent was obtained from all individual participants included in the study.

The analysis was conducted taking the following parameters and data into consideration:

I Histopathological diagnosis:
1. Chronic cholesteatoma of the middle ear,
2. Chronic otitis media with inflammatory granulation;

II Preparation structure:
1. Regular,
2. Irregular;

III Present in the preparation:
1. Matrix,
2. Perimatrix,
3. Not applicable;

IV Present in the preparation:
1. Bacteria,
2. Biofilm,

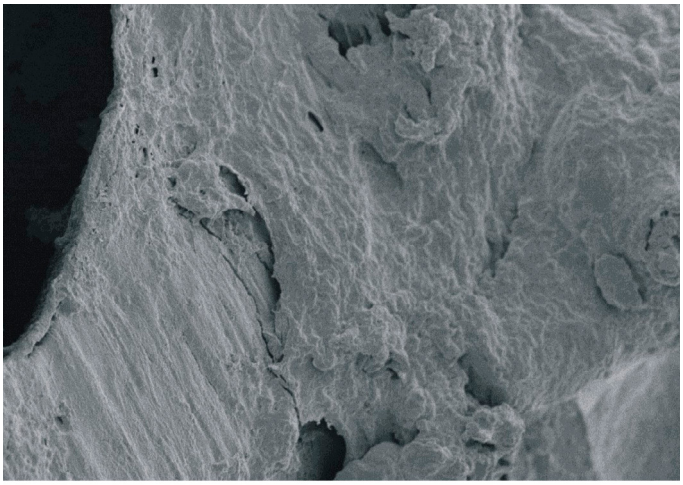

Fig. 4. Inflammatory granulation. Magnification x200. Leukocytes. The Laboratory of Scanning Microscopy.

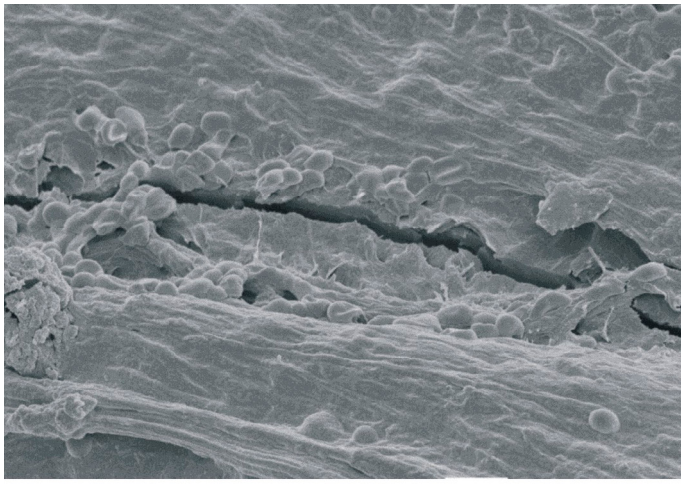

Fig. 5. Inflammatory granulation. Magnification x1000. Leukocytes. The Laboratory of Scanning Microscopy.

3. Fungi,
4. Parasites (Demodex folliculorum),
5. Not found;

V Present in the preparation:

1. Leucocytes,
2. Erythrocytes,
3. No morphotic elements were found.

RESULTS

The analysis of specimens under the Scanning Electron Microscope revealed the presence of cholesteatoma in 60% (12 cases) and 40% (8 specimens) of inflammatory granulation. The structure of cholesteatoma was regular in 50%, and in the second half irregular and even chaotic. In each specimen of cholesteatoma, the matrix and perimatrix were separated. In 7 cases, bacteria in the form of plankton were identified. Bacterial biofilm was observed in two specimens of inflammatory granulation. The analysis of specimens revealed no fungi and no species of Demodex parasites (which mainly concerns cholesteatoma). The influx of inflammatory cells – leucocytes was observed in all specimens of inflammatory granulation and in four cases of acquired cholesteatoma.

The details in microstructure of cholesteatoma and inflammatory granulations in the course of chronic otitis media revealed by means of examination under the Scanning Electron Microscope (SEM) were presented in Fig. 1.–5.

The results of analysis of specimens under the Scanning Electron Microscope (SEM) were collected in Tab. I.

DISCUSSION

According to the literature, acquired cholesteatoma is made up of 5–15 layers of flat cells, and the cells that make up the matrix are arranged in basal, spinous, granular, horny and transparent layers. The cholesteatoma perimatrix is made out of connective tissue (collagen and reticulin fibers) and inflammatory cells (neutrophils, histiocytes, lymphocytes). This structure is associated with a noticeable regularity resulting from the reconstruction, exfoliation and deposition of flat cells [7]. In our work, the regular structure of cholesteatoma depicted under the Scanning Electron Microscope concerned 6 patients with diagnosed chronic cholesteatoma of the middle ear. In the remaining 6 patients, the system was irregular and even chaotic. The observation of irregular matrix structure is consistent with the research carried out by means of the Scanning Microscopy Laboratory by A. Miodonski, who demonstrated a chaotic matrix system in all of the analyzed preparations with diagnosed chronic cholesteatoma of the middle ear [8]. This is due to the fact that the proliferation of keratinocytes in cholesteatoma is much less coordinated compared to normal epidermis, whose pattern is regular. Such surface features suggest abnormal keratosis in the cholesteatoma formation process. The regular keratinocyte pattern found in nearly 42% of the analyzed preparations from patients with chronic cholesteatoma of the middle ear is consistent with the observations made by Youngs and Rowels in a study presented in 1990. The regular structure of cholesteatoma, unlike the irregular one, results in less damage to the bone tissue in the nearest adhesion of the cholesteatoma, which was observed by the authors of this research in a different study [9]. This matter will be a subject of a separate analysis. A conclusion arises that a regular matrix structure and the associated close connection between the keratinocytes reduces molecular permeability of inflammatory cytokines, simultaneously decreasing the destructive activity of cholesteatoma on the bone structures, which can be observed in the case of a chaotic and irregular matrix structure. The similarity of the regular cholesteatoma matrix structure to that of normal skin constitutes, a barrier to the permeability of agents, involved in the complex mechanism of bone resorption in cholesteatoma. Contrasting dependency can be observed in cases of irregular or even chaotic structure of the matrix. Lack of regularity can also be observed in the case of granulation tissue, which in the SEM image presented itself as an irregular tissue mass without detectable regularities and repetitions and with no possibility of distinguishing structural elements. The term granulation tissue was introduced by Theodor Billroth in 1865, indicating that its structure is initiated primarily by fibroblasts.

Tab. I. Chronic cholesteatoma otitis media and chronic granulomatous otitis media – results obtained from Scanning Electron Microscopy.

NO. OF SPECIMEN	RESULTS OF HISTOPATHOLOGICAL EXAMINATION	STRUCTURE OF SPECIMEN: 1. REGULAR, 2. IRREGULAR.	ANALYZED DETAILS: 1. MATRIX, 2. PERIMATRIX, 3. N/A.	PRESENT IN THE PREPARATION: 1. BACTERIA, 2. BIOFILM, 3. FUNGI, 4. PARASITES (DEMODEX FOLLICULORUM), 5. N/A.	PRESENT IN THE PREPARATION: 1. LEUCOCYTES 2. ERYTROCYTES 3. NO MORPHOTIC ELEMENTS.
P1	1	1	1/2	1	3
P2	1	2	1/2	1	1
P3	1	1	1/2	1	3
P4	1	1	1/2	1	3
P5	1	2	1/2	5	1
P6	1	2	1/2	1	1
P7	1	2	1/2	5	3
P8	1	1	1/2	5	1
P9	1	1	1/2	5	3
P10	1	1	1/2	1	3
P11	1	2	1/2	1	3
P12	1	2	1/2	5	3
P13	2	2	3	5	1
P14	2	2	3	5	1
P15	2	2	3	5	1
P16	2	2	3	2	1
P17	2	2	3	2	1
P18	2	2	3	5	1
P19	2	2	3	5	1
P20	2	2	3	5	1

I Results of histopathological examination: 1. Chronic cholesteatoma otitis media; 2. Chronic otitis media with inflammatory granulation.
II Structure of specimen: 1. Regular, 2. Irregular.
III Analyzed details: 1. Matrix, 2. Perimatrix, 3. N/A.
IV Present in the preparation 1. Bacteria, 2. Biofilm, 3. Fungi, 4. Parasites (Demodex folliculorum), 5. N/A.
V Present in the preparation:1. Leucocytes, 2. Erythrocytes, 3. No morphotic elements.

They produce collagen, which is converted outside of the cells into fibres and gives the tissue strength. Apart from that, fibroblasts are also the source of proteoglycans – the basic, jelly-like substance of the extracellular space, which is an indicator for assessing the wound healing tendency and quality. In the case of chronic otitis media with inflammatory granulation, the healing process does not occur as active inflammation (through pro-inflammatory factors) activates the tissue to inflammatory reaction, the final effect of which can be the observed process of transformation into polyps in the middle ear. An active inflammatory process is evidenced by a large influx of leukocytes: neutrophils and lymphocytes, which are sources of pro-inflammatory cytokines (IL-1 a/b, IL-6, IL-8, TNF) as well as the antagonist group composed of anti-inflammatory cytokines (IL-4, -5, -10, -13) [10, 11]. The raised concentration of pro-inflammatory cytokines enhances their direct influence on osteoclasts, increasing differentiation and stimulating their resorption properties. Conversely, it also directly influences osteoblasts, inhibiting their differentiation and activity. All processes lead to the damage of bone tissue and the progression of inflammation. In our analysis, we observed a lymphocyte influx in all granulation tissue preparations indicating active inflammation. In the case of preparations obtained from patients with chronic cholesteatoma of the middle ear, the inflow of inflammatory cells was lower and affected four cases (Tab. I.).

Although SEM imaging is widely used for the identification and characterization of biofilms, no presence of biofilm was determined in any of the preparations in our analysis. However, the presence of bacterial colonies was observed. According to various authors, cholesteatoma tissue is a favorable environment for the formation of biofilms. Lampikoski et al. noted the formation of biofilm in three out of four patients with exacerbation of inflammation during the cholesteatoma process and in three out of five (60%) cases of cholesteatoma. According to Ercan Kaya et al., the frequency of biofilm

observation in patients with cholesteatoma was 61.5%. Chole and Faddis identified biofilms in 16 out of 24 clinical cases of cholesteatoma (66%). The authors of these studies suggested that bacteria can infect the keratin matrix, creating biofilms, which in turn lead to chronic and persistent infections [12, 13]. In our analysis, the lack of biofilm documented in the SEM assessment does not exclude its presence [14]. There is strong evidence for the existence of bacterial biofilms in cholesteatoma. The existence of bacterial biofilms in cholesteatoma may explain the clinical course of exacerbations of ear inflammation in cholesteatoma, i.e. the persistence and recurrence of infection, with the surgical elimination of the cholesteatoma mass being the only effective treatment. Bacterial colonies were observed in both cholesteatoma tissue (7 cases) as well as in patients with chronic otitis media with inflammatory granulation (2 cases). None of the preparations showed any evidence of fungal colonies or parasites (especially in the case of cholesteatoma tissue) living in the epidermis of the Demodex species. The parasites of Demodex species played an unconfirmed role in the pathogenesis and development of acquired cholesteatoma. Based on literature, Demodex folliculorum could take part in development of rosacea, perioral inflammation, blepharitis folliculitis or pityriasis folliculorum. Because of the etiology of acquired cholesteatoma, unrestrained growth and chronic inflammation, there could be multiple other factors that have an influence on its inflammatory activity [15–17].

CONCLUSIONS

1. The regular pattern of the cholesteatoma matrix cells observed in some patients with chronic cholesteatoma of the middle ear reduces the molecular permeability of inflammatory cytokines, concurrently limiting the destructive activity on bone structures;

2. The presence of inflammatory granulation tissue in the middle ear is accompanied by an influx of leukocytes: neutrophils and lymphocytes, which are the source of pro-inflammatory cytokines, the growth of which activates the processes leading to the damage of bone tissue and the development of inflammation;

3. No specimen of acquired cholesteatoma revealed the presence of commensal organisms from Demodex species on the surface of exfoliated human epithelium.

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REFERENCES

1. Emmett S.D., Kokesh J., Kaylie D.: Chronic Ear Disease. Med Clin North Am., 2018; 102: 1063–1079.
2. Boroń A., Wiatr A., Składzień J. et al.: The effect of preserved stapedial superstructure on hearing improvement. Otolaryngol Pol., 2019; 74(1): 1–5.
3. Kim J.M., Lin C., Stavre Z. et al.: Osteoblast-Osteoclast Communication and Bone Homeostasis. Cells., 2020; 9(9): 2073.
4. Othman Z., Mohren R.J.C., Cillero-Pastor B. et al.: Comparative proteomic analysis of human mesenchymal stromal cell behavior on calcium phosphate ceramics with different osteoinductive potential. Mater Today Bio., 2020; 24(7): 100066.
5. Chen X., Wang Z., Duan N. et al. : Osteoblast-osteoclast interactions. Connect Tissue Res., 2018; 59(2): 99–107.
6. Wiatr M., Skladzien J., Strek P. et al.: Chronic Otitis Media with Granulation Is a Poor Prognostic Factor for Hearing Improvement and Development of Intracranial Complications. J Int Adv Otol., 2019; 15(1): 12–17.
7. Galletti B., Gazia F., Freni F. et al.: Chronic Otitis Media Associated with Cholesteatoma in a Case of the Say-Barber-Biesecker-Young-Simpson Variant of Ohdo Syndrome. Am J Case Rep., 2019; 20: 175–178.
8. Sunwoo W., Lee S.Y., Seong J. et al.: Clinical Characteristics of Patients with Cochlear Fistulas Caused by Chronic Otitis Media with Cholesteatoma. J Int Adv Otol., 2020; 16(1): 40–46.
9. Akarcay M., Kalcioglu M.T., Tuysuz O. et al.: Ossicular chain erosion in chronic otitis media patients with cholesteatoma or granulation tissue or without those: analysis of 915 cases. Eur Arch Otorhinolaryngol., 2019; 276(5): 1301–1305.
10. Emmett S.D., Kokesh J., Kaylie D.: Chronic Ear Disease. Med Clin North Am., 2018; 102: 1063–1079.
11. Bayat A., Saki N., Nikakhlagh S. et al.: Ossicular chain defects in adults with chronic otitis media. Int Tinnitus J., 2019; 23: 6–9.
12. Vestby L.K., Grønseth T., Simm R. et al.: Bacterial Biofilm and its Role in the Pathogenesis of Disease. Antibiotics (Basel)., 2020; 9(2): 59.
13. Grønseth T., Vestby L.K., Nesse L.L.: Bioactive glass S53P4 eradicates Staphylococcus aureus in biofilm/planktonic states in vitro. Ups J Med Sci., 2020; 125(3): 217–225.
14. Jamal M., Ahmad W., Andleeb S.: Bacterial biofilm and associated infections. J Chin Med Assoc., 2018; 81(1): 7–11.
15. Türkmen D., Türkoğlu G.: Demodex Infestation in Patients with Rosacea. Turkiye Parazitol Derg., 2019; 43: 194–197.
16. Jacob S., VanDaele M.A., Brown J.N.: Treatment of Demodex-associated inflammatory skin conditions: A systematic review. Dermatol Ther., 2019; 32(6): e13103.
17. Lavy I.: Demodex parasites and chronic blepharitis. Harefuah., 2019; 158: 112–114.

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