

OTOLOGY

Effect of stapes demineralisation on the development of cochlear otosclerosis

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SUMMARY

Objective. The involvement of the inner ear in otosclerosis may lead to the development of cochlear otosclerosis. The aim of this study was to analyse changes in the chemical composition and microstructure of the stapes in the course of otosclerosis compared to healthy stapes.

Materials and methods. This analysis included 31 patients with otosclerosis and 9 patients without otosclerosis. Microanalytical and diffraction techniques were used to assess the elemental distribution and orientation topography of the stapes.

Results. The concentration of Ca^{2+} in the study group was significantly lower in the area of the anterior crus of the stapes than in the posterior crus. A reduction in the $\text{Ca}^{2+}/\text{P}^{3+}$ ratio in the anterior crus was associated with deteriorated bone conduction and tinnitus. Degradation of the stapes microstructure in the area of otosclerotic lesions was observed with scanning electron microscopy.

Conclusions. Bone remodelling is most significant at the closest location to typical otosclerotic lesions with hydroxyapatite porosity and scale-like bone formation according to scanning electron microscopy. There is a relationship between the disturbance of calcium metabolism and the development of clinical symptoms of cochlear otosclerosis.

KEY WORDS: otosclerotic lesions, cochlear otosclerosis, stapes demineralisation, scanning electron microscope, stapes microstructure

Introduction

Hearing loss at any age can significantly reduce quality of life, thus leading to a lack of adaptation and social isolation. A related cause of acquired hearing loss is otosclerosis.

Otosclerosis is the primary disease affecting the bony labyrinth. The essence of the disease is pathological remodelling of the temporal bone, the basis of which involves the abnormal resorption of bone tissue and its remodelling, thus leading to dysplasia. This disease, which occurs only in humans in the temporal bone, is characterised by progressive hearing impairment and tinnitus. The aetiology of otosclerosis is complex, multifactorial and can involve both environmental and genetic factors.

According to the current state of knowledge, the activity of osteoblasts and osteoclasts is not observed in the bony labyrinth in healthy people. During the course of otosclerosis, processes of abnormal resorption and recalcification of the endochondral layer of the temporal bone occur. The endochondral layer is an area that allows small regions of immature cartilage (known as interosseous globules) to be populated by osteoblasts and osteoclasts ¹⁻³.

The disease process may involve all of the bony structures of the ear; however, the initiating lesion is often adjacent to the *fissula ante fenestram* and spreads throughout the vascular canals to the remaining areas of the temporal bone through calcification of the annular ligament and remodelling of the stapes footplate. Involvement of the inner ear in the disease leads to degenerative changes in the spiral ligament, vascular striatum, organ of Corti and cochlear neurons.

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Current surgical treatments (through the use of a prosthesis) restore the mobility of the ossicular chain, but neither eliminate the disease nor inhibit further bone remodelling in temporal bone.

An understanding of the mechanisms affecting stapes bone tissue in otosclerosis will form the basis for further research on conservative treatments, which may be of key importance for people at risk of developing this disease ⁴⁻⁶. The aim of this study was to analyse changes in the chemical composition and microstructure of the stapes in otosclerosis with respect to healthy stapes. The specific objective was to analyse the impact of changes in the chemical composition of bone in otosclerosis on the functions of the inner ear.

Materials and methods

A prospective analysis of patients who underwent surgery from 2020 to 2021 was conducted. The study included 31 patients with otosclerosis (77.5%) and 9 patients without otosclerosis, who constituted the control group (22.5%). Thirty-four women and 6 men were analysed to determine various influential factors. The oldest patient was 61 years old, and the youngest was 32 years old. Mean age was 42 years. Moreover, the group of patients with otosclerosis consisted of 26 women and 5 men aged 22-55 years. The mean age of these patients was 38 years.

Stapes suprastructures that were obtained as medical waste during surgery were analysed by scanning electron microscopy (SEM). Thirty-one stapes from patients with otosclerosis and 9 healthy stapes were included in the study. The healthy stapes constituted the control group; their removal resulted from the use of the infracochlear approach for vestibular schwannoma excision.

In all patients with otosclerosis, typical preoperative otolaryngological diagnostic tests were performed, which consisted of a physical examination, medical history and audiometric diagnosis, including accumetric examinations, tuning fork tests, pure-tone audiometry and impedance audiometry. The threshold values for air and bone conduction were determined by using the Madsen® MIDIMATE® 622 audiometer equipped with TDK 39® headphones. The audiometer met the requirements of the ISO standards for air (ISO0389-1985) and bone conduction (ISO7566-1987).

Pure-tone audiometry was immediately performed before surgery and at 12 months after stapedotomy. The obtained data were analysed according to the guidelines developed by the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology - Head and Neck Surgery ⁷.

SEM examination was performed using a QUANTA 3D FEG scanning electron microscope in low-vacuum mode. A variable vacuum ranging from 0.3 to 0.6 Torr was used

in the tests, with carbon dioxide as the gas. Electron beam accelerating voltages of 15-20 kV, electron beam currents of 5.66, 11.3 and 23 nA and working distances (i.e., the distance from the sample surface to the objective lens pole piece) ranging from 8-14.6 mm were used. A low-vacuum secondary electron detector was used to assess bone surface topography via secondary electrons, and a low-voltage, high-contrast detector was used to record the backscattered electron (BSE) signal.

Qualitative analysis of the chemical composition in areas of the stapes was performed by using an EDS Genesis X-ray detector; PhiRoZet software was used at the quantitative analysis stage.

Chemical composition analysis was performed by using the Raymond Gauvin correction procedure. In addition, we used the variable pressure method, which consisted of measuring two spectra at two different CO₂ pressures (i.e. the first spectrum at a pressure that was twice as high as the second spectrum), after which we extrapolated the maximum values of the individual peaks to zero gas pressure values.

Descriptive statistics were used to describe the characteristics of the studied groups, including the mean, median, standard deviation (SD), first and third quartiles [interquartile range (IQR)] and range. The normality of the distribution of individual parameters that were assessed in this study was checked with the Shapiro-Wilk test. The Mann-Whitney or Kruskal-Wallis U test was used to analyse data with a non-normal distribution. Moreover, the Dunn pairwise test was used to compare individual groups. To determine whether there were differences between the results of particular analysed variables in particular periods (the dependent variables) in the study group, the non-parametric Wilcoxon test or Student t test with Bonferroni correction for multiple testing was used. To investigate the existence of monotonic relationships between the variables, Spearman's correlation coefficient was used. A mixed-effects linear model was used to investigate the relationships between the variables. The level of significance was set at $p < 0.05$.

Results

In the first stage, the chemical composition of the stapes was analysed by comparing the head, anterior crus and posterior crus in the test group.

A significant difference in the Ca²⁺ distribution was observed among the different areas of the stapes (Kruskal-Wallis test, $p < 0.05$) in the study group. In addition, a statistically significant difference in the Ca²⁺/P³⁺ ratio among the analysed areas of the stapes was found in the study group ($p = 0.0481$; Kruskal-Wallis test).

There was no significant difference in the distribution of P^{3+} among the areas of the stapes in patients with otosclerosis ($p = 0.302$; Kruskal-Wallis test). The results are presented in Table I.

The applied Dunn post hoc tests allowed for precise determination of which groups exhibited statistically significant differences. The results are presented in Table II. The concentration of Ca^{2+} was significantly lower in the anterior crus of the stapes than in the posterior crus ($p < 0.05$).

In the next stage, the analysis was performed for the control group, which showed no significant differences in the distribution of Ca^{2+} or P^{3+} among the head, anterior crus and posterior crus (Tab. III). In the next stage of the study, the results obtained for the test and control groups were compared. A significant difference was demonstrated in the distribution of the Ca^{2+}/P^{3+} ratio in the anterior crus of the stapes between patients in the test and control groups ($p < 0.05$; Mann-Whitney U test) (Tab. IV). Patients in the study group had a significantly lower Ca^{2+}/P^{3+} ratio in the anterior crus than patients from the control group. The analysis of the Ca^{2+}/P^{3+} ratio distribution in the posterior crus did not reveal any significant relationships ($p = 0.8018$; Mann-Whitney U test). In the next stage of the study, the relationship between the chemical composition of the stapes and the presence of symptoms typical of otosclerosis, such as tinnitus or sensorineural hearing loss – indicative of cochlear otosclerosis – was evaluated.

In the study group, a significant correlation was observed between the Ca^{2+} level in the anterior crus and the occurrence of tinnitus ($p < 0.05$; Mann-Whitney U test) (Tab. V). Tinnitus patients had significantly lower Ca^{2+} levels in the anterior crus than patients without tinnitus in the study group.

A significant relationship between the occurrence of tinnitus and the duration of disease was demonstrated. The lower Ca^{2+}/P^{3+} ratio observed with increased duration of otosclerosis was significantly more likely in the presence of tinnitus ($p = 0.0027$; Mann-Whitney U test).

A reduction in the Ca^{2+}/P^{3+} ratio in the anterior crus was highly correlated with deteriorated bone conduction (Spearman's correlation coefficient 0.531, $p = 0.03$; Wilcoxon test). In the posterior crus and head, the correlation was low or weak (Spearman's correlation coefficients: 0.028, $p = 0.905$; Wilcoxon test and 0.165, $p = 0.499$; Wilcoxon test, respectively).

However, when considering the posterior crus, there was a significant correlation between the Ca^{2+}/P^{3+} ratio and disease duration in years (Spearman's correlation coefficient -0.474, $p = 0.026$; Wilcoxon test). This correlation was negative and of an average strength. This means that for a longer disease duration, the Ca^{2+}/P^{3+} ratio in the posterior crus was lower.

In patients with otosclerosis, changes in the surface microstructure of the stapes in the area of the anterior crus were noticeable; specifically, degradation of the stapes micro-

Table II. Comparison of Ca^{2+} concentrations among stapes areas in the study group by post hoc testing.

Compared areas	p value
Head - Anterior crus	0.068
Head - Posterior crus	0.923
Anterior crus - Posterior crus	0.014

p: significance level.

Significance level (p) < 0.05 in bold.

Table I. Comparison of the chemical composition of different areas of the stapes in the study group.

Variable	Parameter	Head of the stapes (N = 31)	Anterior crus of the stapes (N = 31)	Posterior crus of the stapes (N = 31)	Test	p value
Ca^{2+}	Mean (SD)	16.5 (7.4)	11.4 (5.4)	17.2 (6.9)	Kruskal-Wallis	0.0245
	Median (IQR)	16 (10.3-19.8)	14.2 (8.6-15.6)	16.9 (12-20.4)		
	Range	7.3-35.1	0.2-17.5	5.3-35.1		
P^{3+}	Mean (SD)	6.8 (4.3)	5.3 (2.7)	6.8 (3)	Kruskal-Wallis	0.302
	Median (IQR)	6.6 (3.5-9.8)	5.4 (4.5-7.2)	6.7 (5.6-8.6)		
	Range	0.9-15.6	0.1-9.5	1.4-15.6		
Ca^{2+}/P^{3+} ratio	Mean (SD)	3.4 (2.4)	2.1 (3.4)	3.3 (2.9)	Kruskal-Wallis	0.0481
	Median (IQR)	2.2 (1.7-4.4)	2 (1.8-2.5)	2.3 (1.6-3.2)		
	Range	1.3-8.9	1.3-17.7	1.1-13.5		

Ca: Calcium; P: Phosphorus; N: number of patients; p: significance level. Significance level (p) < 0.05 in bold.

Table III. Comparison of chemical composition in different areas of the stapes in the control group.

Variable	Parameter	Head of the stapes (N = 9)	Anterior crus of the stapes (N = 9)	Posterior crus of the stapes (N = 9)	Test	p value
Ca ²⁺	Mean (SD)	4.7 (2.2)	14.1 (2.6)	14.6 (2)	Kruskal-Wallis	0.2745
	Median (IQR)	4.9 (3.7-5.7)	14.9 (12.1-16.4)	15.6 (13.6-15.9)		
	Range	1-8.2	9.9-17.1	11.6-17.2		
P ³⁺	Mean (SD)	3 (1.3)	1.6 (0.2)	6.7 (0.2)	Kruskal-Wallis	0.0726
	Median (IQR)	2.8 (2.2-3.9)	1.7 (1.4-1.9)	6.7 (6.6-6.9)		
	Range	1-5	1.2-2	6.3-6.9		
Ca ²⁺ /P ³⁺ ratio	Mean (SD)	1.6 (0.7)	8.7 (2.5)	2.1 (0.3)	Kruskal-Wallis	0.0611
	Median (IQR)	1.3 (1.2-1.9)	8.3 (7.6-8.5)	2.2 (2-2.3)		
	Range	0.7-3.2	5.5-13.5	1.7-2.5		

Ca: Calcium; P: Phosphorus; N: number of patients; p: significance level.
Significance level (p) < 0.05 in bold.

Table IV. Comparison of the chemical composition of the anterior crus between the study and control groups.

Variable	Parameter	Study group (N = 31)	Control group (N = 9)	Test	p value
Ca ²⁺	Mean (SD)	11.4 (5.4)	14.1 (2.6)	Mann-Whitney U	0.3736
	Median (IQR)	14.2 (8.6-15.6)	14.9 (12.1-16.4)		
	Range	0.2-17.5	9.9-17.1		
P ³⁺	Mean (SD)	5.3 (2.7)	1.6 (0.2)	Mann-Whitney U	0.0973
	Median (IQR)	5.4 (4.5-7.2)	1.7 (1.4-1.9)		
	Range	0.1-9.5	1.2-2		
Ca ²⁺ /P ³⁺ ratio	Mean (SD)	2.8 (3.4)	8.7 (2.5)	Mann-Whitney U	0.0198
	Median (IQR)	2 (1.8-2.5)	8.3 (7.6-8.5)		
	Range	1.3-17.7	5.5-13.5		

Ca: Calcium; P: Phosphorus; N: number of patients; p: significance level.
Significance level (p) < 0.05 in bold.

structure was visible, with scale-like structures visible on the bone on SEM (Fig. 1).

In patients from the control group, SEM clearly showed the fibrous structure of hydroxyapatite (which is characteristic of healthy bone tissue in the stapes), with no signs of degeneration (Fig. 2). In otosclerosis, hydroxyapatite porosity was observed by using BSE imaging, which resulted from stapes bone degeneration (Fig. 3).

Discussion

In the present research, the chemical composition analysis confirmed that the main component of the bone structure was

hydroxyapatite with a variable Ca²⁺/P³⁺ ratio. In otosclerosis, bone tissue demineralisation was observed. At the level of biochemical analysis, the microscopic changes were accompanied by changes in the chemical composition, with measurable bone tissue decalcification visible in the immediate vicinity of the otosclerotic foci and significant differences observed relative to healthy bone.

In this study, a significant difference in Ca²⁺ distribution was observed among different areas of the otosclerotic stapes, with the lowest level occurring in the anterior crus, followed by the head and posterior crus (p = 0.0245, Kruskal-Wallis test). Comparative analysis of the Ca²⁺ distribution in the healthy stapes (control group) did not show a significant difference

Table V. Comparison of the chemical composition of the anterior crus in the presence or absence of tinnitus in the study group.

Variable	Parameter	Tinnitus present (N = 20)	Tinnitus absent (N = 11)	Test	p value
Ca ²⁺	Mean (SD)	11.3 (3.5)	18.8 (7.6)	Mann-Whitney U	0.0283
	Median (IQR)	11 (8.4-13.9)	19.1 (15-22.7)		
	Range	7.3-16	7.3-35.1		
P ³⁺	Mean (SD)	5.2 (3.1)	7.6 (4.7)	Mann-Whitney U	0.3802
	Median (IQR)	4.4 (3.4-6.6)	7 (3.8-12)		
	Range	1.6-10.3	0.9-15.6		
Ca ²⁺ /P ³⁺ ratio	Mean (SD)	2.7 (1.5)	3.7 (2.7)	Mann-Whitney U	0.4827
	Median (IQR)	1.9 (1.6-3.9)	2.2 (1.8-4.4)		
	Range	1.5-4.9	1.3-8.9		

Ca: Calcium; P: Phosphorus; N: number of patients; p: significance level. Significance level (*p*) < 0.05 in bold.

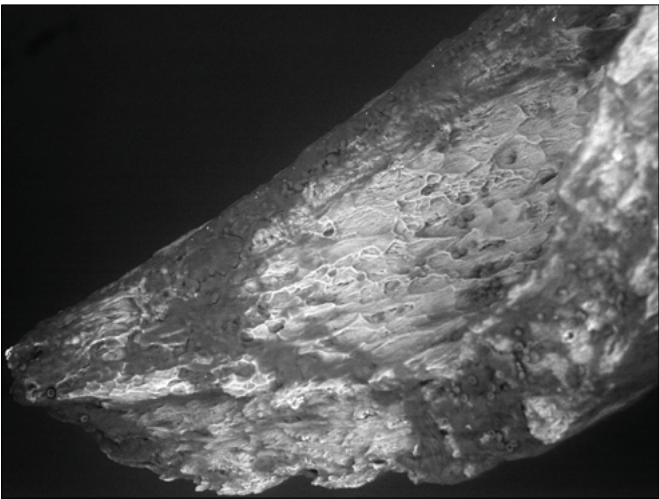


Figure 1. Otosclerosis. Microstructure of the anterior crus of the stapes, showing bone with a scale-like structure of hydroxyapatite on scanning electron microscopy (SEM) using a backscattered electron (BSE) signal; magnification, 1000x.

among the head, anterior crus and posterior crus. There was a significant difference in the distribution of the Ca²⁺/P³⁺ ratio in the stapes between the otosclerosis and control groups. Patients with otosclerosis had a significantly lower Ca²⁺/P³⁺ ratio in the anterior crus than patients in the control group (*p* = 0.0198; Mann-Whitney U test). According to the literature, the Ca²⁺/P³⁺ ratio in normal bone is not always constant over time. Skerry ⁸ believes that the initial amorphous phase of calcium phosphate in healthy bones is gradually and successively converted to octa-calcium phosphate Ca₈(HPO₄)₂(PO₄)₄•5H₂O, tricalcium phosphate Ca₃(PO₄)₂ and hydroxyapatite. These forms

of mineralisation occur only transiently in immature foetal bone; therefore, only the mature form of calcification represented by hydroxyapatite is found in adults ⁸. The bony labyrinth, unlike most cranial bones, which are formed as a result of intramembranous ossification, develops like long bones as a result of the endochondral ossification process. The most suitable chemical model for the main bone mineral phase is Ca₅(OH)(PO₄)₃ hydroxyapatite, containing approximately 5% to 10% CO₃²⁻ groups and approximately 5% to 10% HPO₄²⁻ groups, in the CaHPO₄•2H₂O configuration. In the bony labyrinth, there

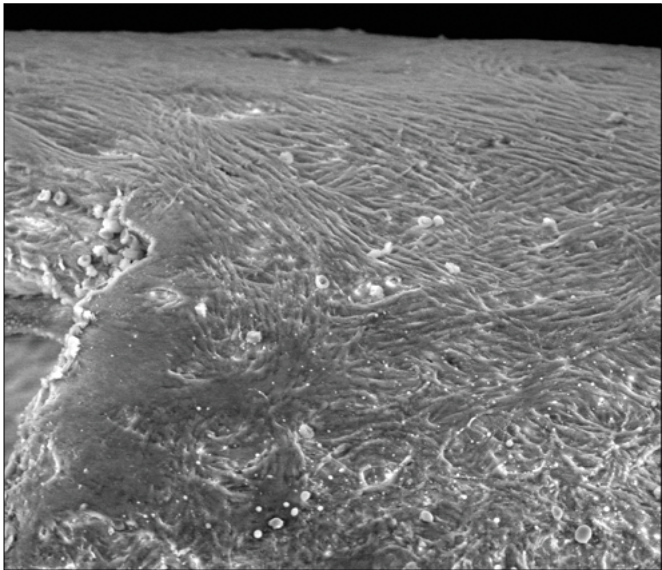


Figure 2. Control group. Microstructure of the anterior crus of the stapes on SEM using a BSE signal; magnification, 1200x.

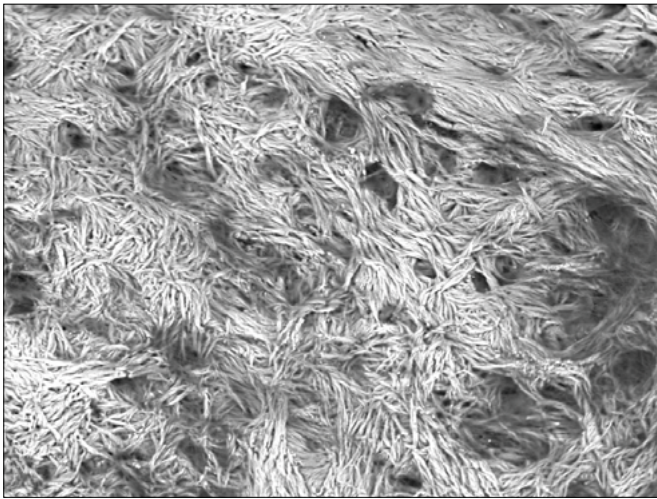


Figure 3. Otosclerosis. Anterior crus of the stapes, showing hydroxyapatite porosity on SEM using a BSE signal; magnification, 1200x.

was no significant remodelling of bone tissue typical of long bones, which is related to the lack of alternating bone resorption and remodelling.

The metabolic rate of the bony labyrinth is approximately 0.13% per year, while in long bones, it is up to 10% per year. This low rate of bone remodelling in the bony labyrinth is due to high levels of osteoprotegerin (OPG), which inhibits osteoclastogenesis and consequent bone resorption. In otosclerosis, the level of OPG in the bony labyrinth is reduced, resulting in a decreased level of bone resorption (otospongiosis phase) in favour of an increased level of remodelling (otosclerosis phase). This causes a change in the chemical composition of the bone tissue and then partial conversion to other related mineral species, such as dahlite or struvite. These transformations are illustrated by the quantitative determination of phosphorus (or phosphates) and calcium, as their relative percentages in each of the quoted mineral species are different. In the assessment of the described processes, it is useful to determine the ratio of calcium to phosphorus ($\text{Ca}^{2+}/\text{P}^{3+}$)^{9,10}.

The change in metabolic activity during the course of otosclerosis has an impact on the degeneration of bone tissue (as shown on SEM by the formation of scale-like bone and porous tissue). The significant differences in the microstructure and chemical composition shown in the analysis concerned individual parts of the stapes in otosclerosis and were considerably different with respect to stapes free from otosclerotic changes.

The process of stapes bone remodelling in patients with otosclerosis was most strongly expressed on the surface of the anterior crus with a typical scaly structure, in contrast to the normal surface of the healthy stapes in patients from the

control group. In the latter cases, SEM showed the typical fibrous structure of hydroxyapatite.

The structure of healthy bone may vary depending on the anatomical location. According to the literature, approximately 8% of PO_4 ions can be replaced in the structure of healthy bone by CO_3 ions; this represents B-type hydroxyapatite. In the case of A-type hydroxyapatite, the OH groups are replaced by CO_3 anions¹¹.

Inner ear involvement in the process of osteodystrophy observed in otosclerosis is described as degenerative changes in the spiral ligament, vascular striatum, organ of Corti and cochlear neurons. In our observations, the progressive decline in the $\text{Ca}^{2+}/\text{P}^{3+}$ ratio in the anterior crus coexisted with deterioration in bone conduction (Spearman's correlation coefficient 0.531, $p = 0.03$; Wilcoxon test). A significant dependence of tinnitus on the duration of disease was also demonstrated. The lower anterior crus $\text{Ca}^{2+}/\text{P}^{3+}$ ratio observed with increased duration of otosclerosis was significantly more likely in the presence of tinnitus ($p = 0.0027$; Mann-Whitney U test). The typical location of otosclerotic foci was the anterior crus; the biochemical manifestation of the ongoing processes was a significant change in the $\text{Ca}^{2+}/\text{P}^{3+}$ ratio in the anterior crus compared to other parts of the stapes.

Changes in the elemental composition and levels of toxic metabolites released during bone remodelling, penetrating the inner ear, seem to negatively affect the inner ear and be responsible for the clinical manifestations of cochlear otosclerosis. Tinnitus intensification has been observed with the progression of otosclerosis and bone remodelling, which, overlapping with hearing loss, deepens the subjective sense of hearing impairment¹².

In a study by Vallejo-Valdezate et al.¹³, a decrease in the $\text{Ca}^{2+}/\text{P}^{3+}$ ratio of the stapes similar to that in otosclerosis was observed in Van der Hoeve syndrome (VDHS). Despite the aetiological differences, both diseases are related to changes in the bony labyrinth and progressive sensorineural hearing loss. While VDHS is caused by a disorder of type I collagen synthesis, it has been postulated that collagen II autoimmunity is present in otosclerosis¹³.

The available reports, similar to our observations, explain the biochemical changes observed in otosclerosis with the probable transformation of hydroxyapatite into tricalcium phosphate and insufficient mineralisation of the collagen matrix¹⁴.

The influence of bone tissue demineralisation on the function of sensory cells of the inner ear has been widely discussed in the otologic literature. Sensorineural hearing loss accompanying demineralisation is explained by the accumulation of Ca^{2+} in inner hair cells. Numerous reports have indicated a relationship between the reduc-

tion in bone tissue density and hearing impairment. Ji Yoon et al. showed that postmenopausal osteoporosis and the related disturbance of calcium metabolism favour the occurrence of sensorineural hearing loss¹⁵. Moreover, Groschel et al. observed the effects of changes in calcium levels with age on the occurrence of sensorineural hearing loss, which also occurred in the upper levels of the auditory pathway¹⁶.

According to research by Xingqi Li et al., the reduced Ca^{2+} concentration in the perilymph mediated by reductions in the Ca^{2+} concentration in inner hair cells has a protective effect on hearing when exposed to noise¹⁷. During the otosclerotic process, bone decalcification promotes the accumulation of Ca^{2+} in inner hair cells.

Sensorineural hearing loss is also significantly more frequent in cases of hypoparathyroidism due to the disturbance of calcium metabolism. Adverse effects on the inner ear are explained by abnormal levels of calcium in the inner ear fluid and/or a direct effect of vitamin D deficiency on the inner ear^{18,19}.

Numerous studies have indicated that a reduced level of vitamin D, in addition to disturbed Ca^{2+} metabolism and abnormal microcirculation in the cochlea, are factors influencing the development of sensorineural hearing loss²⁰. Decreased serum Ca^{2+} and vitamin D levels, which are often associated with osteoporosis via increases in bone metabolism, are considered independent risk factors for hearing impairment. Furthermore, decreased vitamin D levels appear to reduce auditory sensitivity by altering Ca^{2+} metabolism and microcirculation in the cochlea²¹.

The accumulation of Ca^{2+} (with an increase in its concentration in the perilymph mediated by increases in the concentration of glutamate) leads to inner hair cell damage²². In inner hair cells, an increase in intracellular Ca^{2+} causes the mobilisation of synaptic vesicles and the exocytotic release of glutamate at the base. The release of glutamate modulates the activity of auditory nerve fibres by activating specific receptors, including the ionotropic glutamate receptors AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor), NMDA (N-methyl-D-aspartate receptor) and KARs (kainic acid receptors), as well as metabotropic receptors. Outer hair cells react completely differently to changes in their membrane potential. Their membranes contain a protein (prestin) that changes conformation with the membrane potential and forces the cell length to change at acoustic frequencies²³.

Increased Ca^{2+} activity via the $\text{CaV}1.2\text{L}$ -type calcium channel in the auditory ossicles and ear capsule through bone remodelling affects the auditory and vestibular functions of the inner ear and underlies the pathophysiology of otosclerosis and other osseous disorders of the ear²⁴.

The current analysis complements the observations of other authors, thus demonstrating a significant role of demineralisation of the stapes and disturbances in calcium ion metabolism in the development of hearing loss in cochlear otosclerosis.

Conclusions

Stapes bone remodelling is most significant in the closest location to typical otosclerotic changes (i.e., in the fissula ante fenestram). There is a relationship between the disturbance of calcium metabolism in otosclerosis and the development of clinical symptoms of cochlear otosclerosis. As the otosclerotic process advances in the stapes, the porosity of hydroxyapatite resulting from bone tissue degeneration and scale-like bone tissue formation increases, as observed by SEM.

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Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

MW, KN, AW: conception; MW, AW: design; MW, RB, KN, AW: data collection; MW, RB, KN, AW: analysis; MW: funding; MW, RB, AW: literature; MW, KN: review; MW, AW: writing.

Ethical consideration

The research was approved by the Institutional Bioethics Committee of Jagiellonian University, Kraków, Poland (approval no. 1072.6120.311.2020). The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from each participant/patient for study participation and data publication.

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