WEB OF SCIENTIST: INTERNATIONAL SCIENTIFIC RESEARCH JOURNAL
ISSN: 2776-0979, Volume 4, Issue 8, August, 2023

INVESTIGATION OF STRUCTURAL CHANGES IN BONE TISSUE BY OPTIMAL METHODS IN CHRONIC PURULENT OTITIS MEDIA IN PATIENTS IN STATIONARY CONDITIONS

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Annotation
Fragments of the bone tissue of the temporal bone were studied during reconstructive-sanitizing interventions in chronic purulent otitis media by light and electron microscopy at various times of the disease. A quantitative analysis of the degree of structural changes in bone tissue in chronic inflammation at the cellular and tissue levels after histomorphological examination with microwave decalcification was carried out. The disposition of studying decalcified off-white interweave representatives put together it accomplishable to contradistinguish the individualism of off-white retrogression in weathers of long-standing pussy inflammation, to catalogue the processes of remodeling and transfigurement of off-white tissue. For the fundamental time, the compartmentalisation of modifications of morphologic substitutions of the material off-white in long-standing instigative cognitive semantics at contradistinctive intervals of the affliction in patients with long-standing pussy otitis media is presented.

Keywords: bone tissue, reconstructive-sanitizing surgery, chronic purulent otitis media.

Introduction

Chronic diseases of the middle ear remain the leading problem in otorhinolaryngology, which has medical and social significance, consisting in the potential danger that the inflammatory purulent process of the temporal bone creates for the patient's life, and providing social guarantees for people with professional

unfitness for hearing loss is a significant economic burden for the state [1-4]. According to the average statistical data, the incidence of chronic purulent otitis media (HCSO) averages 23.9 cases per 100 thousand population [5-7]. V.T. Palchun (2009) noted that any patient with a chronic inflammatory process should be considered as a candidate for surgical treatment, and sanitizing surgery for HCSO is only a stage before reconstruction of various structures of the middle ear [8]. In the structure of ear diseases, HCG accounts for 27.2%, only 5.7—7% of patients are assisted in hospitals, of which 45-54.2% of patients undergo surgical treatment, more than 70% have various types of sanitizing operations on the middle ear [5]. Otogenic complications, which occur in 3.2% of patients when mortality reaches 16.1%, become important. As a rule, the recurrent destructive process of the temporal bone, which occurs in 24-63% of patients with HCSO, leads to fatal outcomes. Bone resorption in HCG with cholesteatoma is detected in 78.8% of cases, without — in 8.2% of cases and is caused not by a special activity of the matrix, but by an inflammatory reaction of the subepithelial tissue due to the activity of enzymatic processes [5, 9]. Inflammatory-regenerative processes in the previously formed open trepanation mastoid cavity proceed for a long time with the formation of immature granulations, scars and exudation, which often leads to repeated sanitizing surgical interventions [10-15]. Summarizing the above, we can conclude that the chronic inflammatory process in the middle ear with the risk of complications requires studying the structures of the bone tissue of the temporal bone for the correct choice of treatment method, reducing the traumatic effect of the sanitizing stage of surgery, including during reconstruction using implantation materials. Pathomorphological changes of the mucous membrane in chronic inflammation are described in many well-known scientific papers, but to date, the features of structural changes in bone tissue as a factor in the prognosis and outcome of reconstructive-sanitizing operations have not been studied, which determines the need to study this issue [16-19].

The purpose of the study. To study the features of structural remodeling of bone tissue in chronic purulent otitis media by light and electron microscopy.

Задачи исследования:

1. To study and substantiate optimal methods of decalcification of temporal bone tissue after reconstructive-sanitizing operations for light and electron microscopy.
2. To analyze the results of pathogenetic transformation of bone structures in chronic purulent inflammation of the mucous membrane of the middle ear at various times of the disease.

Materials and methods of research. The study used a proven method of intraoperative mucoperiostomy of the medial wall of the tympanic cavity and retrotimpanal sections and bone tissue of the middle ear [17] in 95 operated patients with HCG aged 12-30 years who gave voluntary informed written consent to participate in according to the data from the accounting and reporting documentation of the clinical bases of the Department of Otorhinolaryngology Samarkand Regional Multidisciplinary Children's Hospital for the period 2020-2022 years . Distribution of patients by type of surgical intervention: reconstructive operations were performed in 29 (30.5%) patients, in 51 (53.7%) — reconstructive-sanitizing, in 15 (15.8%) - sanitizing. The selection of the material for the study was carried out as follows: 8 samples — for choosing the method of bone decalcification, 39 — for histomorphological analysis of the inflammatory process in the mucous membrane and bone tissue of the middle ear, and 48 — for histomorphological study of structural changes in bone tissue by light microscopy. To select the method of bone tissue decalcification, blocks of biopsy material (No. 8) were examined — fragments of bone tissue that were decalcified according to the generally accepted scheme, according to current regulations and standards [12]. It is well known that when working with bone material, it is important to preserve the histomorphological picture of the drug, to obtain high-quality sections with preserved tinctorial properties. Since the study of bone tissue makes it difficult to contain a large amount of mineral substances, acid and acid-free decalcifiers were used for light and electron microscopic examination of samples. The wiring of non-decalcified bone material is practically not used, since it is uninformative [2]. 39 samples were examined for histomorphological analysis of the inflammatory process in the mucous membrane of the middle ear. Fixing, wiring and filling of the material was carried out according to the generally accepted scheme, according to the current regulations and standards [5]. Tissue sections were fixed in a 10% solution of neutral formalin and poured into paraffin. Serial plane-parallel sections with a thickness of 5 microns were made from each paraffin block. Tissue sections were stained with hematoxylin and eosin, according to Van Gieson, followed by JENAMED-2 light microscopy at magnification of 200, 400. To assess the pathomorphological intraoperative condition of the mucous membrane of the middle ear, a semi-quantitative pathomorphological technique based on the basic criteria for assessing the inflammatory-reparative process was used.

To assess the histiomorphology of decalcified temporal bone tissue in a histioprocess at the light-optical level, 48 samples were studied according to the criteria: the severity of the cleavage line and the Havers canal, the clarity of concentric and insertion plates, changes in the tinctorial properties of the nucleus and processes of

osteocytes [8]. During electron microscopic examination of bone tissue, the following criteria were evaluated: integrity of the osteocyte plasmalemma, degree of cytoplasm vacuolization, integrity of membrane organelles, degree of chromatin condensation and destruction, integrity of the walls of lacunae, safety of processes and channels in which the processes pass, and striation of collagen fibrils. The severity of each criterion was evaluated in points: in the absence of severity of the criterion — 0 points, mild severity — 1 point, with good severity of the criterion — 2 points. Then the scores were summed up and the highest value was found [11]. With traditional methods of decalcification using various acids , we obtained: indistinctness of lacunae boundaries, osteocyte cytoplasm is partially destroyed, departs from the edge of lacunae, split collagen protofibrils and numerous dense granules, disappearance of striated collagen fibrils, etc.. In the future, the method of acid-free decalcification of bone tissue with ethylenediaminotetraacetic acid salts under the action of microwave radiation in a histioprocessor (Sakura Tissue-Tek VIP) was used [9]. The meshes were studied in a Libra-120 transmission electron microscope (Carl Zeiss & MT, Germany) with an UltraScan 950 digital SSCCD camera (4 megapixels) in the magnification range of 1200-20 000.

Statistical processing of the research results was carried out using the standard licensed software package of applied statistics SPSS 11.0 for Windows. In the study, the conditions for the normality of the distribution were not met, therefore, nonparametric Mann—Whitney criterion was used to calculate statistically significant differences in values in groups where $n>30$, with a significance level of $p < 0.05$.

Results and discussion

During the morphological examination of 39 samples of the mucous membrane and bone tissue of the middle ear, biopsies were compared according to the following parameters: the activity of the inflammatory process (assessed by the intensity of lymphoplasmocytic, macrophage and granulocytic infiltration), the level of fibrotization, the intensity of vascularization, edema of the stroma of the mucous membrane of the middle ear and retrotimpanal sections, as well as slugphenomen, thrombosis in the capillaries of the stroma. The activity of chronic inflammation of chronic otitis media: inactive inflammation was detected in 15 patients, low inflammation activity in 11, moderate inflammation activity in 4, and high inflammation activity in 9 patients ($p=0.001$).

Morphological examination of 48 samples of bone material of the middle ear taken intraoperatively revealed signs of structural changes of varying severity. As a result of histomorphological examination, the bone ultrastructures of the temporal bone in

chronic inflammation were destroyed regardless of the duration of the patient's HCG disease (see table). Our study showed that in the histomorphological picture of chronic low-active inflammation of the mucous membrane of the tympanic cavity with fibrosis, granulations and reactive changes in the integumentary epithelium, the bone tissues of the middle ear had the most pronounced changes in the ultrastructures of cells and intercellular substance, as a result of which the structure of bone tissue was disturbed with the death of specialized bone structures and the absence of remodeling processes during implantation otosurgery. Based on a semi-quantitative analysis of the degree of structural changes in bone tissue in chronic inflammation at the cellular and tissue levels by microwave decalcification with light and electron microscopy, classification criteria for bone tissue transformation were identified according to three main variants: 1st variant — osteonecrosis with pronounced destruction of bone tissue — in the absence of osteoblasts and the death of some osteoclasts with weakly expressed inflammatory infiltration; fibrous tissue, as a rule, with the presence of chronic weakly active inflammation. Pathomorphological picture — there is no reconstruction of bone tissue against the background of sluggish granulation inflammation with lacunar resorption, which subsequently leads to the replacement of mastoid process cells with connective tissue. Option 2 — osteoproliferation with reactive bone thinning — bone tissue with the presence of inflammatory infiltration, proliferation of osteoblasts; fibrous tissue with signs of chronic inflammation. Pathomorphological picture — there is a restructuring in the bone tissue with a predominance of bone formation, altered sclerosed cells to varying degrees, in which signs of excessive bone formation and rarefaction of bone tissue are combined. The 3rd variant is osteosclerosis with vascular resorption of bone tissue, while pronounced hemorrhages in the inter—trabecular space are actively replaced by fibrosization processes in the absence of osteoblasts, osteoclasts and inflammatory cell infiltration. Pathomorphological picture — hemorrhages and bone resorption stimulate the growth of granulation and scar tissue with subsequent ossification.

Distribution of patients in groups by prescription of the disease

Table № 1

Term	Research Group	Study Group (%)	p
Up to 1 year	4	4,2	0,109
Up to 2 years	5	5,3	
Up to 5 years	9	9,5	
Up to 10 years	15	15,8	
Up to 15 years	12	12,6	
More than 20 years	50	52,6	
Total	95	100	

Conclusions

The results of the study allow us to reveal one of the mechanisms of the pathogenesis of chronic inflammation in the disturbed processes of osseointegration of bone tissue structures in patients with HCG and its purulent complications. To verify the degree of transformation, histomorphological examination with light and electron microscopy is an objective method for analyzing the structural features of the bone structure of the temporal bone at the tissue and cellular level. The method of studying decalcified bone tissue samples makes it possible to differentiate the nature of bone degeneration in conditions of chronic purulent inflammation, to register the processes of remodeling and transformation of bone tissue. The study of demineralized preparations of the temporal bone showed that the destruction of bone tissue with a pronounced violation of the main histomorphological structures is important to take into account for the correct choice of treatment method, reducing the traumatic effect of the sanitizing stage of surgery, including during the auditory-improving stage of reconstruction using implantation materials in patients with HCSO. The proposed classification criteria for structural changes in bone tissue in chronic inflammation of the mucous membrane of the middle ear, despite the complexity and high cost of the research methods used, are of practical importance for objectifying the characteristics of inflammation in destructive forms of HCG and its purulent complications.

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