ORIGINAL ARTICLE

DIFFERENTIAL-DIAGNOSTIC INFORMATIVENESS OF THE MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF PAROTID SALIVARY GLAND CYSTS

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ABSTRACT

The aim: To conduct a general analysis of the results of the study of the morphological and immunohistochemical structure of cysts of the parotid salivary glands.
Materials and methods: Our study is based on the application of generally accepted, additional and special methods of examination, which concerns 21 patients who underwent surgical intervention to remove cystic formations of the parotid salivary gland.
Results: It was established that there are 2-3 HLA-DR+ cells per 100 epithelial cells located in the basal and subbasal layers in the form of their continuous ribbon and their moderate infiltration of tissues within the acinar epithelium. In the epithelium, CD3+ cells were also detected in the number of 1 to 7 per 100 epitheliocytes and they were the most numerous, along with HLA-DR+ cells. Instead, the presence of CD4+ and CD20+ cells was not detected in the epithelium, unlike the subepithelial layer, where they occupied significant areas. In turn, the infiltration of CD8+ cells of the epithelial layer was established in the amount from 1 to 7 per 100 epitheliocytes. A moderate number of them was also determined subepithelially, and they were single directly in the cyst wall.
Conclusions: Immunohistochemical study of the structural components of cystic formations is this is the direct way to establish the nature of the redistribution of immune cells in it, which is very important when conducting differential diagnosis in difficult and doubtful cases.

KEY WORDS: parotid salivary gland, cyst, morphological structure, immunohistochemistry

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INTRODUCTION

Cystic formations more often originate from the small salivary glands (56%), less often from the sublingual (35%), parotid (5%) and submandibular (4%). They develop mainly in young and middle-aged people, and their formation may be associated with a partial or complete cessation of the outflow of secretion. The leading causative factors of impaired patency of the ducts of the glands can be their blockage with a mucous plug, obliteration due to the presence of an inflammatory process both in the gland itself and in the case of direct traumatic damage to it and adjacent soft tissues, obstruction by a stone of the duct system, cicatricial narrowing or external compression by a tumor. There are opinions that some cysts of the large salivary glands can develop during embryogenesis and originate from the epithelium that was detached or formed from an additional rudimentary duct, therefore, nowadays, the questions of their etiology and pathogenesis remain debatable [1, 2].

Most often, cysts of large salivary glands are recognized by the clinical picture of their development, but in order to establish the correct diagnosis, it is necessary to carry out additional differential diagnosis using special research methods. Ultrasound examination, cystography, sialography, MRI in the contrast mode is usually performed to clarify the size, structure and position of the cyst, its connection with the salivary gland, especially when there is a suspicion of malignancy of the formation. An aspiration puncture biopsy with subsequent biochemical and cytological examination of the cellular composition of the contents and a study of the morphological structure of the cyst itself after its removal is of great importance in confirming the diagnosis [3-5].

Cysts of the parotid salivary glands are always represented by cavity formations that arise as a result of obturation of their excretory ducts and are filled with liquid contents. Clinically, they are manifested by the presence of a dense or dense-elastic painless protrusion, a slow

increase in size, and sometimes can lead to difficulty in swallowing. Diagnostic measures involve the use of a classic version of generally accepted methods, which include the collection of complaints, medical and life anamnesis, analysis of data from visual, clinical, additional and special examination methods [6, 7].

However, it should be noted that the classic method of histological verification of parotid gland cysts is sometimes controversial, and fine-needle aspiration cytology (FNA-fine-needle aspiration), which is most often used and is associated with a high frequency of false-negative results. In such cases, the use of coronal biopsy under ultrasound control is a more invasive procedure than FNA, but safer and more diagnostically effective. In turn, the frozen biopsy section provides better species specificity than FNA, but it also has a number of disadvantages and cannot be considered as the main diagnostic tool. Therefore, recently, more and more attention of scientists is paid to the newest methods, which include the immunohistochemical variant of studying the layers of cystic membranes, which determines the relevance of this scientific research [8].

THE AIM

The aim of the research is to conduct a general analysis of the results of the study of the morphological and immunohistochemical structure of cysts of the parotid salivary glands.

MATERIALS AND METHODS

Our study is based on the application of generally accepted, additional and special methods of examination, which concerns 21 patients who underwent surgical intervention to remove cystic formations of the parotid salivary gland on the basis of the maxillofacial surgery department of the ME "Poltava Regional Clinical Hospital named after M.V. Sklifosovsky" of Poltava Regional Council in the period from 2015 to 2022. During the examination of the patient, the size, shape of the cyst, turgor, color of the skin in the area of its location, the presence of an inflammatory reaction, consistency, reaction of the regional lymph nodes were evaluated.

With the help of a ultrasound-controled puncture biopsy, material was obtained from the formation for further study of the cellular composition of the punctate after staining the smear according to Romanovsky-Giemza with the determining of the activity of α-amylase in it, and the presence of which allows to establish the fact of the presence of a cyst of the salivary gland, and this was carried out according to the Karavey`s method. Ultrasound examinations were carried out using scanners "HDI 5000", "Dornier AI 5200", "Aloka 630", with linear or convex sensors and a radiation frequency of 7.5 or 10 MHz. Multifrequency hydrogel acted as an intermediate medium. In tricky cases and when there were doubts or suspicions about the malignancy of the formation, 5 patients underwent an MRI examination according to the standard method.

Sections for studying the morphological structure of the cyst shell with a thickness of 6-8 μm were prepared from 21 blocks on a MBS-2 microtome using standard classical methods. They were fixed on glass slides and stained with hematoxylin-eosin. Van Gieson's picrofuchsin staining was also applied and the PAS-reaction was performed. [9, 10]. The results were documented using a light microscope "Olympus BX-41" (Japan) with a photomicroscope and a set of licensed image processing programs. Part of the results were captured by filming with a microscope using a video camera "Panasonic WV-CP410/ G" (Japan) on the basis of the department of pathological anatomy of the National Medical Academy of Postgraduate Education named after P.L.Shupyk.

In addition, biopsies of the cyst walls were placed in a 6% solution of carboxymethylcellulose (Sigma, USA), which was frozen in liquid nitrogen, and sections 5-7 μm thick were made on a microtome-cryostat. The immunohistochemical study consisted in the determination of subpopulation markers of immunocytes localized in different layers of 21 shell biopsies. At the same time, HLA-DR+, CD3+, CD4+, CD8+, CD20+ immune cells were studied for quantitative and qualitative characteristics, using monoclonal antibodies to these molecules produced by "Sorbent". Immunohistochemical study on cryostat sections was carried out according to the previously described method [11-14].

In order to determine the shares in percentages, a generally accepted statistical method was used [15].

RESULTS

Of the 21 patients selected for further in-depth research, 18 patients (85.7%) were referred by communal city polyclinics, and the remaining 3 (14.3%) were referred by primary care institutions. At the pre-hospital stage, dental surgeons diagnosed 17 people (80.9%) as acute or chronic lymphadenitis of the submandibular region, and only 4 patients (19.1%) had a diagnosis corresponding to the nosological form of the disease.

Regarding the duration of the disease, the patients presented ambiguous data: 9 patients (42.8%) noted that the onset of the disease reaches more than 1 year,

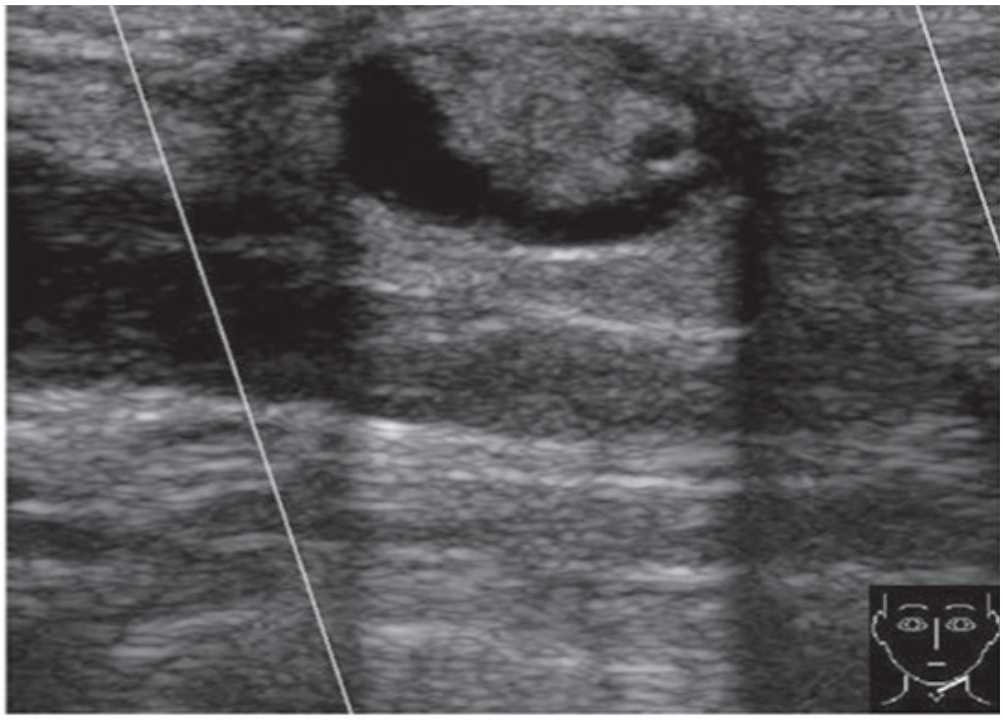

Fig. 1. Echogram of the parotid salivary gland. The presence of a cystic formation measuring 3x4 cm in the lower pole of the gland with hypoechoic content and finely dispersed inclusions was determined.

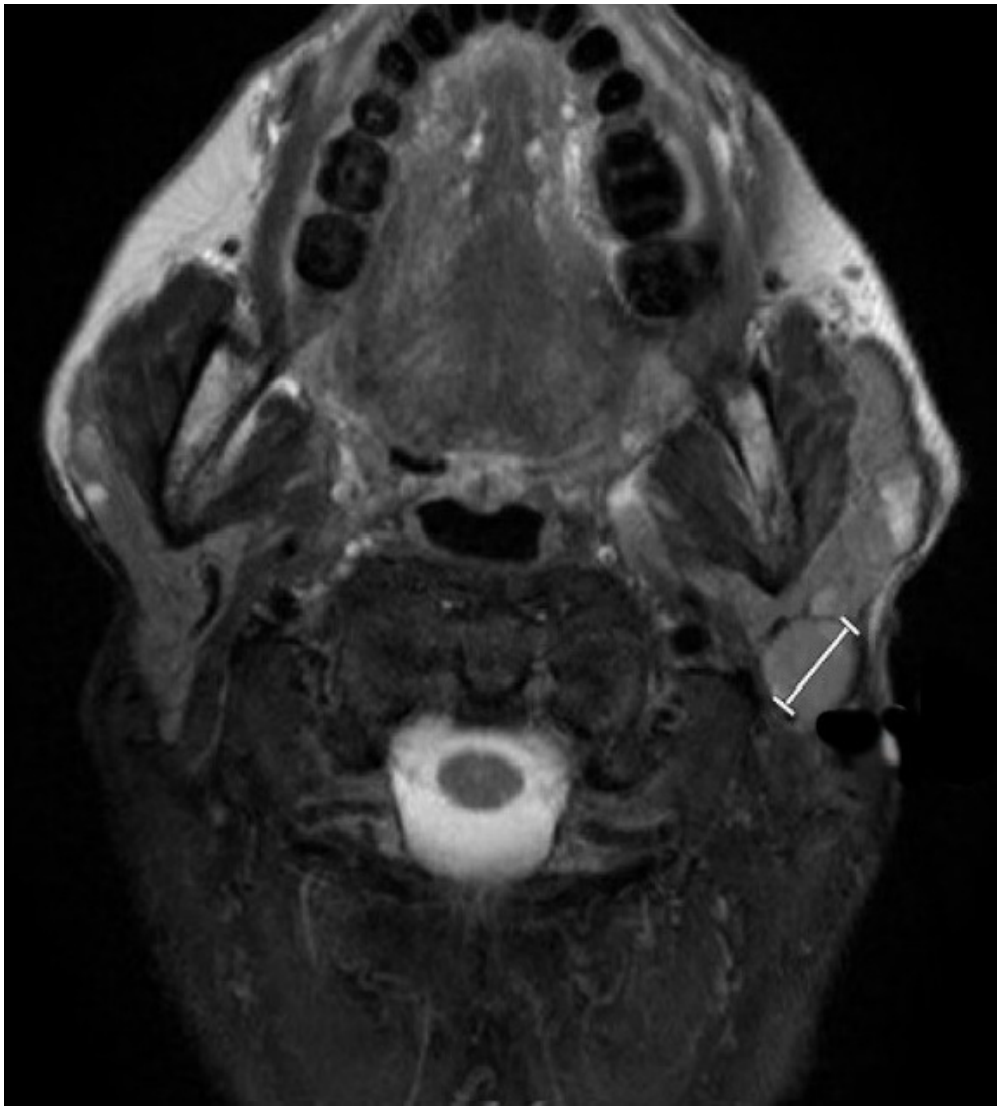

Fig. 2. Image of a parotid salivary gland cyst on an MRI slice. Within the location of the lower pole of the gland, a formation measuring 2x2 cm is visualized.

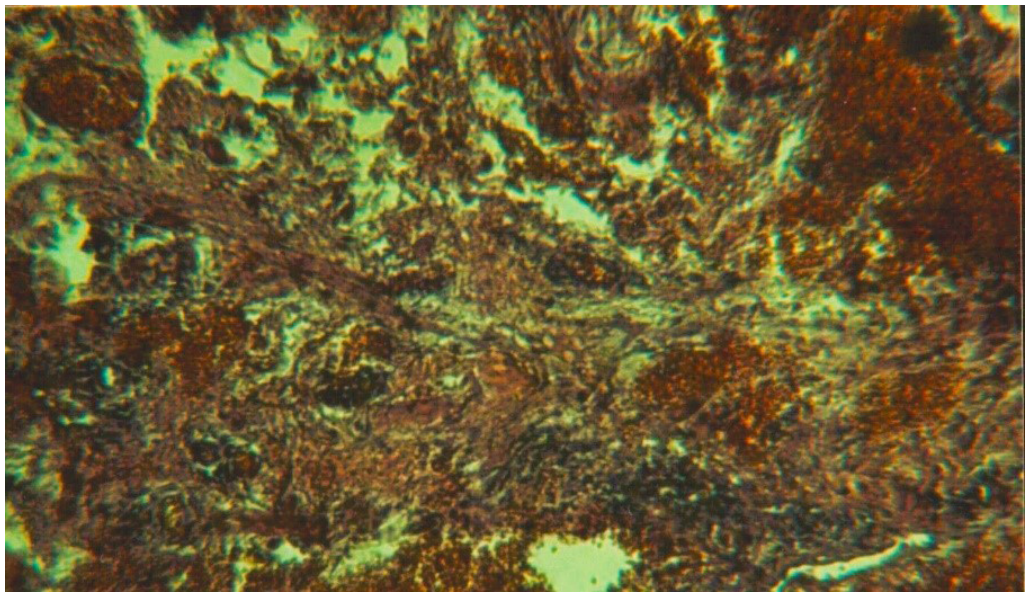

Fig. 3. Positive PAS-reaction throughout the thickness of the epithelial layer of the cyst.

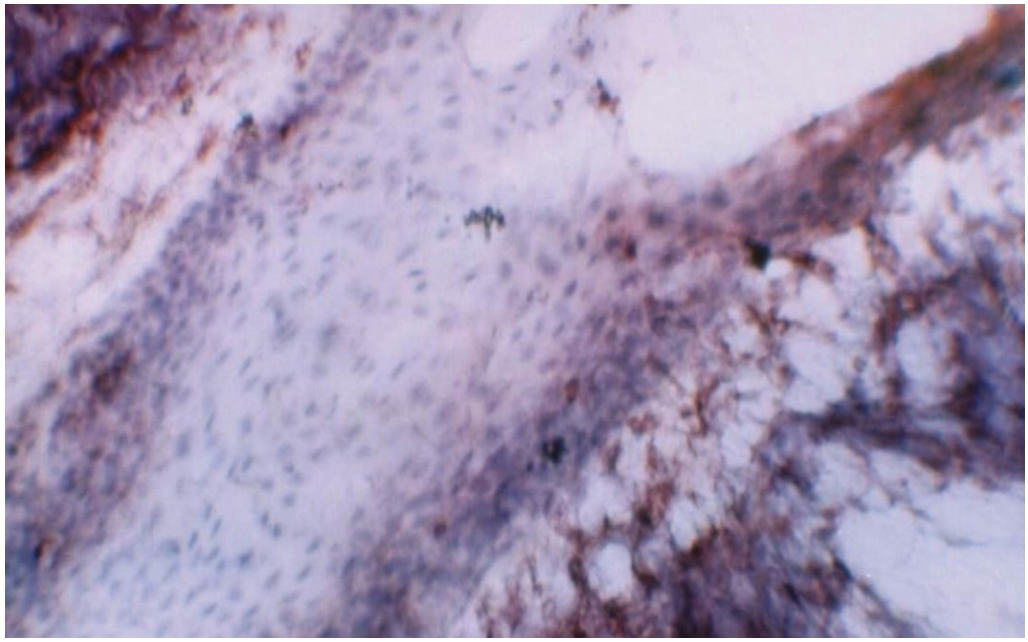

Fig. 4. The presence of HLA-DR+ cells within the acinar epithelium.

7 patients (33.4%) indicated a period of 6 months, 3 patients (14.3%) indicate that the formation appeared about 3 months ago and 2 patients (9.5%) noted the appearance of swelling no more than a week ago. The vast majority of patients sought medical help from specialists of related profiles at their place of residence, and usually they were prescribed for symptomatic treatment.

During the visual assessment of the condition of the maxillofacial region of the patient, asymmetry was determined due to the presence of a tumor formation in the upper third of the lateral region of the neck. In most patients (14 people, 66.7%) the formation was oval in shape, and in 7 patients (33.3%) it was rounded. The skin above the swelling was of a normal shade and it was taken in a strip.

Variation in the size of the cysts was also noted: in 15 patients (71.4%), they were from 2 to 4 cm, in 6 cases (28.6%) - from 4 to 5.5 cm. We determined the consistency of the formation in 1 case (4.8%) as dense, in 17 patients (80.9%) as dense-elastic, and in 3 (14.3%) as elastic. During the palpation examination of the cysts, the contour surface was smooth in all of them, the formations were painless, not fused with the surrounding tissues, but slightly limited in mobility. The reaction of regional lymph nodes was determined in 11 patients (52.4%) both by palpation and during ultrasound examination.

According to the results of an ultrasound examination, these formations had clear contours of a hyperechoic shell with a thickness of 1-2 mm, as well as a hypoechoic structure and finely dispersed inclusions (Fig. 1).

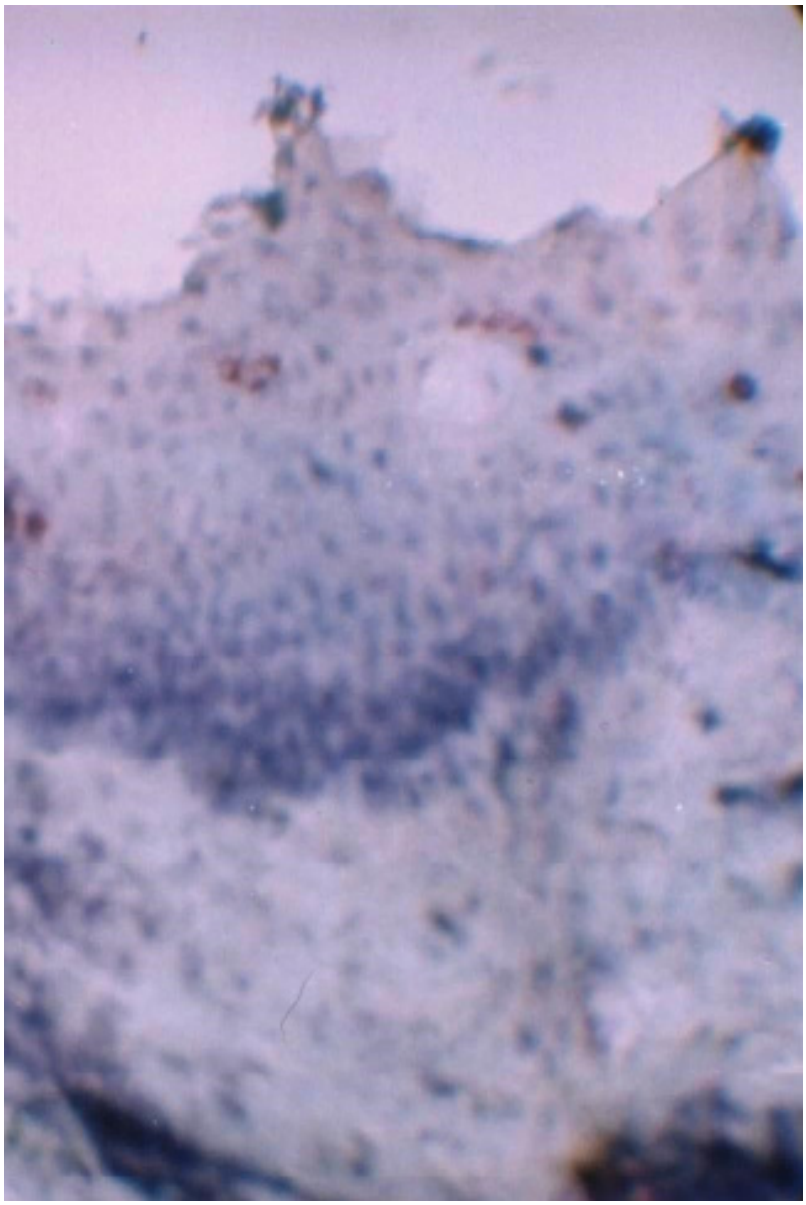

Fig. 5. Presence of CD3+ cells in the epithelial layer.

During fine-needle aspiration under ultrasound control, a transparent liquid of a slightly yellowish color with mucus impurities was obtained. Cytological examination of the punctate revealed the presence of a large number of flat epithelial cells in the form of layers and stratums, single erythrocytes, lymphocytes and cholesterol crystals against the background of structureless masses. The result of the biochemical analysis of the aspirate for α-amylase activity was positive in all cases, which is a fundamental difference of this type of cyst.

In patients who underwent an MRI examination, the presence of tumor-like formations of various sizes with clear contours and reduced echogenicity of the contents was established in the lower parts of the gland (Fig. 2).

Operative intervention was carried out according to classical methods, while general anesthesia was used in 16 patients (76.2%), and 5 patients (23.8%) were operated on under local anesthesia. Wound healing occurred by primary tension. All cystic formations were sent for further morphological and immunohistochemical examination.

The morphological structure of the walls of the cyst of the parotid salivary gland consisted of a connective tissue membrane, an epithelial lining, which was represented by a multi-layered flattened epithelium with basal and spiny cells, in some places the phenomenon of parakeratosis and papillary growths were observed. Areas of round cell infiltration with lymphoid substance and glandular epithelium acini were located under the epithelium layer, which were separated by thickened connective tissue membranes. It should be noted that in some areas the epithelial lining was absent. When the material was stained and the PAS-reaction was

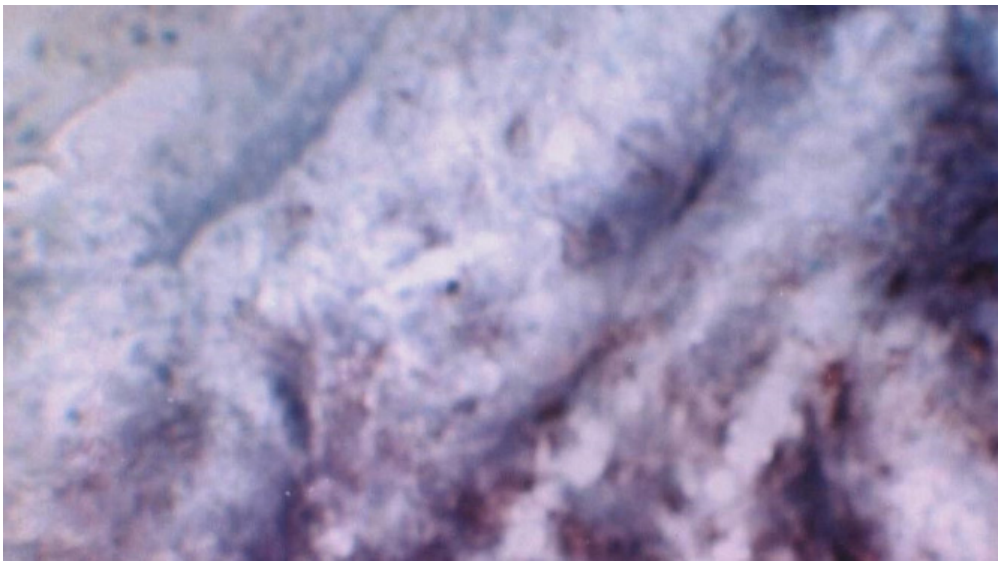

Fig. 6. Presence of CD8+ cells in the epithelial layer

performed, a positive result was established throughout the thickness of the epithelial layer (Fig. 3).

With the help of the performed immunohistochemical studies, it was possible to determine the presence of 2-3 HLA-DR+ cells per 100 epithelial cells, and a positive reaction in the form of a continuous band of HLA-DR+ inclusions was observed in the basal and subbasal layers, or moderate infiltration by them was observed within the acinar epithelium (Fig. 4).

In addition, CD3+ cells were determined in the epithelium in the number of 1 to 7 per 100 epitheliocytes and they were the most numerous, along with HLA-DR+ cells (Fig. 5). The presence of CD4+ cells along with CD20+ cells in the epithelium was not detected, unlike the subepithelial layer, where they occupied significant areas.

In turn, the infiltration of CD8+ cells of the epithelial layer was established in the amount from 1 to 7 per 100 epitheliocytes. A moderate number of them was also determined subepithelially, and they were single directly in the cyst wall (Fig. 6)

DISCUSSION

From a scientific point of view, further research on establishing the nature of the redistribution of immunocompetent cells in different layers of the parotid salivary gland cyst can clarify some aspects of the embryonic laying and indicate its dysontogenetic origin. Taking into account the fact that the facial area is formed from three germ layers, there is a favorable situation for the formation of benign formations in this anatomical area in the process of embryogenesis.

In all cases, the final diagnosis is determined by the morphological structure of the cyst, but this is not always a guarantee of accurate verification, therefore, in difficult and doubtful cases, there is an urgent need for additional involvement of the newest technologies, which include the immunohistochemical method. In different layers of the cystic membrane, certain regularities of their location are observed and directly in the epithelial layer they are represented by small numbers of HLA-DR+, CD3+ and CD8+ cells; the subepithelial layer is intensively infiltrated with HLA-DR+, CD3+ and, to a lesser extent, CD4+, CD8+, and CD20+; HLA-DR+, CD3+ and a small number of CD8+ cells are located around the acini. In the connective tissue of the capsule, immunocytes are not represented.

Shynkevych VI et al. (2021) also used an immunohistochemical method to determine the density of CD68+ and CD163+ cells as preliminary morphological equivalents of different subpopulations of Mφs. The molecules CD68 and CD163 are scavenger receptors that contribute to the polarization program of these cells. In turn, Avetikov DS et al. (2020) determined the influence of polymorphism of collagen type I alpha-2 gene (COL1A2) (rs42524) on the formation of scar tissue localized in the head and neck region. As a result of these studies, it became possible to indirectly indicate the activation of the protective reaction of the skin to physiological scarring and dosed formation of scars in different areas of the head and neck [16-18].

Immunohistochemical studies have proved to be particularly informative in hematological and oncological practice, thanks to which it became possible to verify rare tumors of various origins. Thus, the research of Magda Zanelli et al. (2021) found that immunohistochemical expression of LMO2 together with some morphological clues can help identify cases of T-LBL in the background of M/LNs-Eo, stating that this group

of disorders can be easily underdiagnosed due to both rarity and proteiform clinical presentation [19].

Andrew M Bellizzi, M.D. (2022) in his review "An Algorithmic Immunohistochemical Approach to Define Tumor Type and Assign Site of Origin" gave answers to a whole series of questions, but he paid special attention to the morphological and immunohistochemical study to determine special risk groups from the point of view of diagnostic considerations regarding keratin broad spectrum of action/CD45/S-100-"triple negative" neoplasm [20].

Unfortunately, there is too little information in the literary sources, which makes it impossible to carry out a detailed comparative comparison of the results we obtained. The general clinical characteristics of parotid salivary gland cysts given in our work do not differ from the existing classical ones, in contrast to the information obtained when applying additional and special research methods, especially regarding the establishment of features of redistribution of immunocompetent cells in different layers of the cystic membrane. This makes it possible to supplement scientific data on the role of immunocompetent structures in the formation of immunological potential, and, accordingly, on the strength of the immune response to external and internal factors at the level of organs, systems and pathological formations, which was the focus of attention in our previous publications [7,12].

CONCLUSIONS

Thus, our research proves the importance of the implementation of the latest technologies and their high informativeness when studying the immunocompetence of individual layers of the wall of parotid salivary gland cysts. Directly, the immunohistochemical method makes it possible to establish the type of redistribution of immune cells in them, which is very important when conducting differential diagnosis in difficult and doubtful cases.

In general, establishing the characteristics of the distribution of cells in tissues can serve as a highly informative test when specifying the nosological form of the disease in complex and doubtful cases, and can also be suitable for predicting the likelihood of an inflammatory component and the possibility of malignancy of certain structural elements of the cystic membrane.

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Conflict of interest:
The Authors declare no conflict of interest.

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