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CLINICAL, MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL PARAMETERS OF EPIDERMOID CYSTS IN THE NECK

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The article presents the features of the clinical course of epidermoid cysts in the neck in 27 patients and the morphological and immunohistochemical characteristics of their 20 biopsy samples. The diagnostic informative value of the method of immunohistochemical research in the study of immunocompetence of various layers of the cyst envelope is determined. It was established that the number of immunocompetent cells in the epithelial, subepithelial and connective tissue layers differs, and certain patterns of their location and redistribution are traced. Based on the comparative analysis of the obtained data, it was stated that the location of cystic formations of different nosological forms in this part of the neck is possible. The similarity of the clinical picture and the unambiguity of some results of routine, additional examination methods often lead to diagnostic errors, making the issue of their differentiation highly relevant to scientists and practising dental surgeons.

Key words: cyst, formation, neck, immunocompetence, cyst envelope.

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КЛІНІКО-МОРФОЛОГІЧНІ ТА ІМУНОГІСТОХІМІЧНІ ОСОБЛИВОСТІ ЕПІДЕРМОЇДНИХ КІСТ ШИЇ

У статті наведені особливості клінічного перебігу у 27 пацієнтів, морфологічних та імуногістохімічних характеристик їх 20 біопатів епідермоїдних кіст ший. Визначена діагностична інформативність методу імуногістохімічного дослідження при вивченні імунокомпетентності різних прошарків оболонки кісти. Встановлено, що кількість імунокомпетентних клітин в епітеліальному, субепітеліальному і сполучнотканинному шарах різняться і прослідковуються певні закономірності їх розташування та перерозподілу. На підставі порівняльного аналізу отриманих даних констатовано, що в цій ділянці ший можливе розташування кістозних утворень різних за походженням нозологічних форм. Схожість клінічної картини та однозначність деяких результатів рутинних, додаткових методів обстеження доволі часто призводить до діагностичних помилок, роблячи питання їх диференціації вкрай актуальним як для науковців, так і для практикуючих хірургів-стоматологів.

Ключові слова: кіста, утворення, ший, імунокомпетентність, оболонка кісти.

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An epidermoid cyst is a cyst of the skin, the inner surface is usually lined with multi-layered squamous epithelium, and horny scales represent its contents. It also occurs at any age of patients. It is mainly localized on the head, torso and upper extremities. Macroscopically, it is a tumour-like formation of round shape, soft consistency with a size of 5 to 40 mm in diameter or more. Usually, the skin over the cyst does not gather in a strip. In cases of secondary infection, it can be hyperemic. The epidermoid cyst is

displaced along with the surrounding tissues, painless on palpation. The diagnosis is established on the basis of the clinical picture and histological examination data [9, 10].

Among the cysts of the lateral part of the neck are bronchogenic, dermoid, epidermoid, and cysts of ectopic salivary glands. During the examination of patients with this pathology, puncture biopsy under the control of ultrasound is often used as a generally accepted routine technique, and in a difficult situation, MRI is performed. In the preoperative period, the cellular composition of the biopsy specimen and its biochemical components are most often used. In the postoperative period, morphological examination of the layered structure of its wall is mandatory. Unfortunately, in basic monographs on this topic and even in modern periodical scientific publications, comprehensive information on the role of immunocompetent cells in the formation of the protective potential of cysts of the lateral neck is not fully covered [3, 5, 6].

Despite the lymphoepithelial theory of the origin of lateral cysts in the neck, which explains their formation from the lymphatic system with the participation of epithelium of different types, we have no strong evidence of the composition and presence of individual pools of lymphocytes. There is currently an immunohistochemical method of research to study this component, the diagnostic informativeness of which is not in doubt for either scientists or practitioners [1, 4, 8].

The purpose of the study was to establish the clinical manifestations of the epidermoid cyst of the lateral part of the neck and to establish the immunohistochemical features of all layers of its envelope.

Material and methods. For the set purpose, the general examination methods included a collection of anamnesis regarding the life and illness of 27 patients, 20 of them underwent surgery with our participation. Clinical studies were performed based on the Department of Maxillofacial Surgery of the Poltava Regional Clinical Hospital from 2014 to 2019.

When examining the patient, attention was paid to the comparison of intact tissues and on the affected side, the size of the formation and the severity of visual changes in this area were established. We evaluated the shape (oval, round), colour and skin turgor at the cyst projection, determined the clarity of its contours, location relative to the sternocleidomastoid muscle, consistency, and adhesion to surrounding tissues, and the direction of its displacement. The presence and severity of reactive changes in regional lymph nodes were established. To make a differential diagnosis, a puncture biopsy of them and the formation were performed at the initial stage of the examination [11, 12, 13].

Given this fact, we selected 27 biopsy samples with epidermoid cysts, 21 % (27/129) of the total number of people with cystic lesions in this location and the peak incidence at 21–35 years of age. Among them were 12 men, 44 % (12/27) and 15 women, 55 % (15/27). In 59 % of patients (16/27), the formation was localized on the left, and in 41 % (11/27) on the right.

Ultrasound examinations were performed on patients on the basis of the Municipal Enterprise CCCH of Poltava City Council with the help of scanners HDI 5000, Dornier AI 5200 and Aloka 630, with linear or convex sensors and a radiation frequency of 7.5 or 10 MHz. The intermediate medium was a multi-frequency hydrogel.

6–8 μm sections were prepared from 20 blocks on an MBS-2 microtome using conventional histological methods. They were fixed on slides and stained with hematoxylin-eosin. This method involves using a chemical complex formed by aluminium ions and oxidized hematoxylin. Picrofuxin Van Gieson's staining was also used, and the PAS reaction was performed. [4, 5]. The results were documented using a light microscope Olympus BX-41 (Japan) with a photomultiplier and a set of licensed programs for image processing. Some of the results were documented by shooting from a microscope with a Panasonic WV-CP410/G (Japan) video camera based on the Department of Pathological Anatomy of the P.L. Shupyk National Medical Academy of Postgraduate Education.

Part of the biopsies of the cyst walls was placed in a 6 % solution of Carboxymethyl Cellulose (Sigma, USA), frozen in liquid nitrogen and made 5–7 μm cryostat sections on a microtome cryostat. Immunohistochemical examination on cryostat sections was performed as described earlier [1]. HLA-DR⁺, CD3⁺, CD4⁺, CD8⁺, and CD20⁺ immune cells were studied for quantitative and qualitative characteristics using monoclonal antibodies to these molecules produced by Sorbent, Russia.

The statistical method was used to determine percentages [2].

Results of the study and their discussion. Among patients who applied to a specialized hospital, 52 % (14/27) were referred to primary care facilities and 48 % (13/27) to municipal city clinics. Doctors diagnosed them with acute serous lymphadenitis of the submandibular area in 33 % of cases (9/27), chronic sialadenitis of the submandibular salivary gland in 30 % of patients (8/27), and only in 37 % (10/27), the diagnosis coincided with that established by us during a comprehensive in-depth examination.

During the examination, 59 % of patients (16/27) focused on swelling in the upper third of the neck, and 41 % of patients (11/27) indicated the presence of a formation in the submandibular region. 56 %

of patients (15/27) associated the occurrence of initial manifestations of the disease with hypothermia, 22 % of patients (6/27) noted the stressful situation and heavy physical exertion, and 22 % of patients (6/27) could not determine the likely provoking factor.

The total duration of the disease varied. Thus, 30 % of people (8/27) noted the appearance of swelling on the lateral surface of the neck within a week to 6 months, and 37 % of patients (10/27) indicated the duration of the disease from 1 to 2 years. The existence of formation for more than 3 years was noted by 26 % of patients (9/27). That is, the vast majority of them sought specialized help in the long term from the appearance of the first clinical manifestations of the disease, which is very alarming.

The long-term existence of the disease, its polymorphic clinical manifestations prompted patients to turn to different doctors in their specialty. Sometimes they were self-medicating, most often with compresses and anti-inflammatory drugs. Among the narrow specialists who prescribed pharmacological drugs were a physician, oncologist, ENT doctor, and dental surgeon. The prescription of antibacterial drugs in 7 % of cases (2/27) and antifungal drugs in 14 % of patients (4/27) remained traditional. Topically, 19 % of patients (5/27) recommended compresses with dimexide, and in 60 % of cases (16/27), no action was taken, even after the experts' recommendations.

Locally, during visual examination of patients, neck asymmetry of varying severity was determined due to tumour formations in the upper third of the lateral surface of the neck. The cysts were oval in 63 % of patients (17/27) and round in 37 % (10/27). The colour of the skin over the site of swelling remained the usual colouring in all cases, and it was taken in a fold. Relative to the sternocleidomastoid muscle, the ratio of neck cysts was variable: in 33 % of cases (9/27), they were located under the muscle, in 18 % above the muscle (5/27), for 38 % of patients (16/27), their location was characteristic in front of the muscle, and in 11 % (3/27) they were localized behind it.

The cyst size also varied: from 2 to 4 cm in 44 % of patients (12/27), from 4 to 6 cm in 30 % (8/27), from 6 to 8 cm in 15 % (4/27), from 8 to 10 cm in 11 % of patients (3/27). In 48 % of cases (13/27), the consistency of the formation was determined as soft-elastic; in 33 % (7/27) as elastic, in 15 % of cases (4/27), it was dense-elastic, and in 11 % of patients (3/27), it was dense. On palpation, the contouring surface was smooth, painless, and not soldered to the surrounding tissues. The response of regional lymph nodes was determined in 48 % (13/27) both by palpation and by the use of ultrasound diagnostics.

In this case, the formations were visualized as hypoechoic structures with clear contours, and fine inclusions were determined in the middle of the cystic cavity. The walls of cysts had a thickness of 1 to 2 mm in 70 % (19/27) of patients, and 2 mm or more in 30 % of patients (8/27).

Aspiration of their contents by fine-needle puncture under ultrasound control and its subsequent visual assessment revealed an amorphous-turbid mass of yellow-white or mucous substance characteristic of this type of cyst with the formation of a shiny film on the surface of the drop. Single red blood cells, lymphocytes, and cholesterol crystals were identified in cytograms. In some places, foci of structureless masses, remnants of destroyed cells, scales, and "bare" nuclei were traced. Against this background is a large number of squamous epithelial cells. They were located separately and in the form of layers and stratums, which confirmed the epidermoid cyst.

A biochemical study of samples for α -amylase activity showed negative results in all cases, which allowed for immediately differentiating salivary cysts. The study of clinical analysis of blood and urine did not reveal significant deviations from the control parameters.

All patients underwent surgery according to the classic options, of which general anesthesia was used in 78 % of patients (21/27), and local anesthesia was performed in 22 % (6/27). There were no complications during the surgery, as in the postoperative period. All cystic formations were sent for morphological examination.

Microscopic examination of the wall of the cyst stained by Van Gieson's revealed dense connective tissue located just below the epithelial layer in 49 % of cases (12/27), in 33 % of patients (9/27) it was with signs of hyalinosis, and in 22 % (6/27) it had a loose structure.

In 48 % of cases (13/27), the epithelial part of the cyst membrane was defined with narrow slits, partially filled with squamous epithelium. This indicated the remains of pharyngeal grooves and blood vessels were found in only 37 % of cases (10/27), which stated in total its ectodermal origin.

HLA-DR⁺ round cells up to 3–5 per 100 epitheliocytes were detected directly in the epithelium. Under the epithelium, there was an alternation of zones with both intense and moderate infiltration. Against the background of the dense connective tissue fibres, we also determined elongated abundant HLA-DR⁺ cells in the form of foci with manifestations of infiltration (fig. 1).

CD3⁺ cells quantity in the epithelium ranged from 4 to 10 per 100 epithelial cells with localization throughout its thickness. Subepithelially their moderate presence was noted with the formation of denser

foci. In the connective tissue capsule, they were represented by small clusters (Fig. 2). In addition, single CD4⁺ cells were found in the epithelium and other areas of the epidermoid cyst wall. They have presented to CD3⁺ cells.

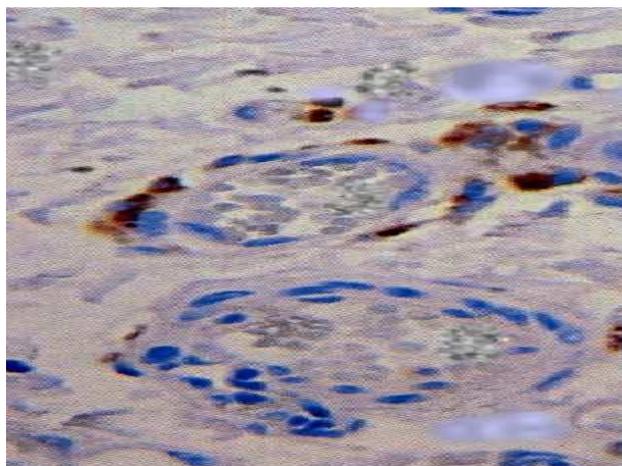


Fig.1 Subepithelial infiltration of HLA-DR⁺ cells. mcAB – anti-HLA-DR, contrast – Mayer's hematoxylin. Magnification x400.

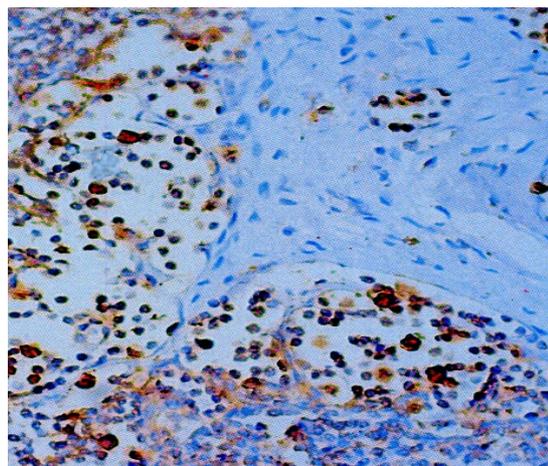


Fig. 2 CD3⁺ cells in the subepithelial layer of the wall of the epidermoid cyst. Mayer's hematoxylin contrast. Magnification x400.

CD8⁺ cells were found selectively in the epithelial layer, and their number only in some areas was 3–5 per 100 epitheliocytes. Subepithelially and in the connective tissue capsule, they repeated the location patterns inherent in CD3⁺ cells (fig. 3).

Single CD20⁺ cells were also found in the epithelium. Subepithelially, there were separate but quite extensive cells without clear restrictions. There was also a pronounced immunopositive reaction along the contour of these cells (fig. 4). No cells were found in the tissues of the CD20⁺ envelope itself.

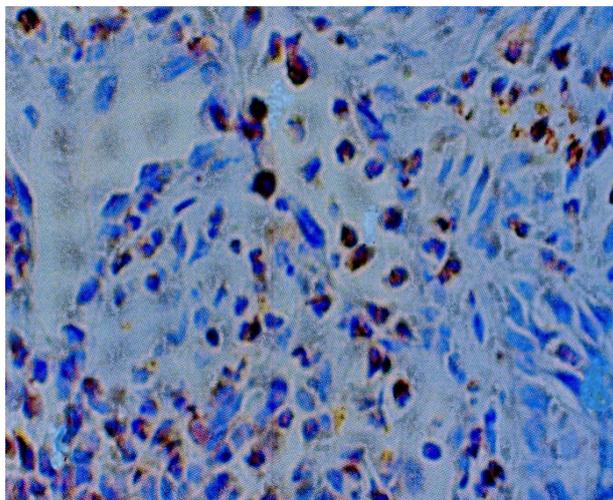


Fig. 3 CD8⁺ cells epidermoid cyst wall cells. Mayer's hematoxylin contrast. Magnification x400.

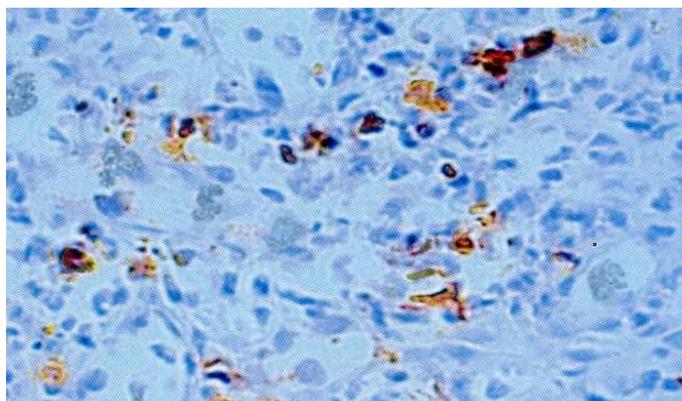


Fig. 4 Foci of cell infiltration in CD20 cells⁺ Mayer's hematoxylin staining. Magnification x400.

From a scientific point of view, further research to establish the nature of the redistribution of immunocompetent cells in different layers of the epidermoid cyst may clarify some aspects of its occurrence. Given the fact that the face is formed from three embryonic leaves, there is a favorable situation for the formation in the process of embryogenesis of benign formations of dysontogenetic nature. In most cases, the final diagnosis is determined by their morphological structure, but given their routine, they are not always a guarantee of accurate verification in doubtful cases. This requires the involvement in examining the latest technologies, including the immunohistochemical method [5, 7]. Under such conditions, such studies should be carried out necessarily taking into account age aspects to establish the role of certain pools of immunocompetent cells in the formation of structural components of epidermoid cysts in the neck, which can determine the features of their clinical manifestations in different periods of life and the intensity of growth. The lack of clear correlations between clinical manifestations, their morphological structure and the nature of the content make it impossible to obtain comprehensive information that can give a detailed answer to some questions. At the

same time, in various layers of a cystic envelope of an epidermoid cyst, certain regularities are traced. The presence of HLA-DR⁺ round cells up to 3–5 was established in the epithelial layer, and CD3⁺ cells ranged from 4 to 10 per 100 epitheliocytes. The number of CD8⁺ cells in some areas reached 3–5 per 100 epitheliocytes; selectively, there were single CD20⁺ cells.

In the subepithelial layer, zones of HLA-DR⁺ cells alternation with both their intensive and moderate infiltration were defined. Moderate infiltration of CD3⁺ cells with the formation of denser cells was noted. Subepithelially and in the connective tissue envelope, CD8⁺ cells repeated the patterns of location inherent in CD3⁺ cells. CD20⁺ cells were located in separate extensive foci without an explicit restriction, and a pronounced immunopositive reaction was traced along their contour.

Against the background of dense connective tissue fibres, the presence of elongated abundant HLA-DR⁺ cells was established in the form of foci, sometimes with manifestations of infiltration. No CD20⁺ cells were found directly in the connective tissue of the cyst wall. The general clinical characteristics of epidermoid cysts of the lateral surface of the neck and its architecture do not differ from those covered in the existing classical publications [4, 10]. However, we could not establish the peculiarities of the redistribution of immunocompetent cells in the various layers of the epidermoid cyst envelope, making it impossible to compare the obtained results for a given period.

Conclusion

Thus, our study proves the importance of implementing the latest technologies in the study of the representation of immunocompetent cells of the layers of the wall of the lateral cysts of the neck of different origins. This allows us to establish the nature of the distribution of immune cells, which is very important for the differential diagnosis in complex or doubtful cases and testifies to the effectiveness and high informativeness of this technique. In addition, if necessary and with the right tactical approach, the immunohistochemical research method may be suitable for predicting the likelihood of acute inflammation and the possibility of malignancy. This requires improvement in the technique of puncture biopsy to remove all layers of the cystic wall. This methodology can be used in everyday clinical practice as a minimally invasive diagnostic and prognostic test.

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