

SECTION 3. DENTISTRY

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3.1 Laboratory methods of research in the diagnosis of oral mucosa diseases. Cytological method

Diseases of the oral mucosa of both adult and children's populations occupy an important place in dental practice [107-110]. Clinical studies show that pathological changes in the oral mucosa are often manifestations of infectious diseases, allergic conditions, and consequence of disorders in the work of various organs and systems in children with concomitant pathology [111-120]. In such cases, the establishment of a final diagnosis requires consultations with doctors of various specialties, and additional research methods [121-127].

Recently, the frequency of manifestations of viral infection in oral cavity has significantly increased among the children's population of Ukraine [112]. It is known that oral mucosa is often the entrance gate for the penetration of the virus into the body. So, according to the WHO, 65-90% of adults and children of the planet are infected with the herpes virus [111]. The growing number of cases of an atypical clinical course of a viral infection in the oral cavity among children requires paediatric dentists to search for new diagnostic approaches. Analysis of the results of microscopic examination of material taken directly from the surface areas of the lesion elements is one of the most informative and accurate methods for diagnosing diseases of the oral mucosa.

Often, inflammatory processes in the oral cavity have a different origin and similar clinical manifestations. Since the results of microscopic (cytological and bacterioscopic) studies are necessary to establish a final diagnosis. In such cases, complex treatment, which is based on the results of cytological diagnostics, can more effectively influence the course of the disease [116, 126].

The results of cytological study of oral mucosa was analysed. Dental examination of patients of different ages was carried out at the department of paediatric

therapeutic dentistry and prevention of dental diseases of the Bogomolets National Medical University (NMU) from 2014 to 2021.

Microscopic examinations were carried out in the clinical laboratory of the dental medical centre of Bogomolets NMU.

Cells taken from the oral mucosa and the surface of the lesion elements (erosion, ulcers, blisters, cracks) were used as the material for cytological studies. In cases of prolonged damage, the material was collected from different parts of erosion due to possibility of formation of morphologically different cells on their its peripheral.

The collection of cytological material was carried out by the method of obtaining dab-imprint, dab-reimprint and dab-scraping. Fixation and staining of the cytological preparations (180) was performed according to the Romanovsky-Gimza method. Dab-imprints were evaluated using light microscopy («МБП-1» [biological worker microscope -1], immersion lens (90), eyepiece 10).

Diagnosis of diseases of the oral mucosa requires the use of various laboratory methods: microscopic (cytological, bacterioscopic), bacteriological (cultural), virological, molecular biological (DNA-polymerisation method), immunological, allergological and general clinical.

Usually, oral mucosa is covered with stratified scaly non-keratinised epithelium. The cytological preparation of intact oral mucosa is characterised by the presence of large epithelial wing cells, a small nucleus and a pale blue cytoplasm. The cells of epithelium are tightly adjacent to each other. Single white blood cells are present in the visual field in Fig. 1.

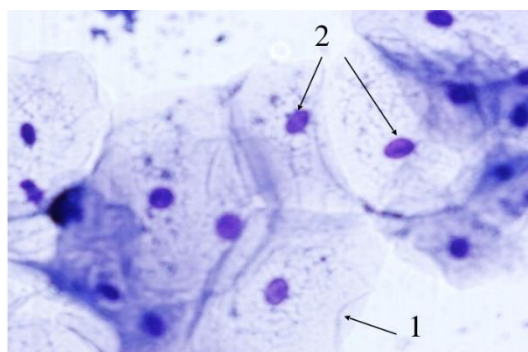


Figure 1. Photo dab-imprint from the surface of the non-keratinised epithelium of oral mucosa (norm: 1 - epithelial wing cell, 2 - nuclei of epithelial cells).

The epithelium of the oral mucosa is keratinised in areas of increased mechanical stress (gums, back of the tongue, the line of contact of the teeth on oral mucosa of the cheeks, hard palate). This is also the norm. Enucleated epithelial wing cells (having lost the nucleus) are found in cytological preparation from these areas.

The nonspecific acute inflammation of the oral mucosa caused by opportunistic bacterial microflora of the oral cavity is characterised by the presence of significant qualitative changes in epithelial cells. Epithelial cells are in the stage of degeneration, numerous inclusions, vacuoles appear in the cytoplasm in Fig. 2.

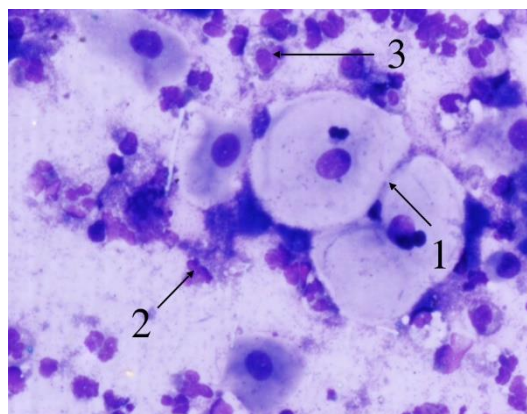


Figure 2. Photo dab-imprint of the oral mucosa in acute nonspecific inflammation, the initial stage: 1 - epithelial cells of the surface layer are located in groups, 2- the remnants of cellular elements, 3- a small number of white blood cells.

The progression of nonspecific inflammatory process in the oral mucosa is accompanied by the process of destruction of a significant number of cells. All visual fields in the dab-imprint are covered with detritus, remnants of cellular elements. There are a large number of microorganisms, the presence of a significant number of white blood cells in Fig. 3.

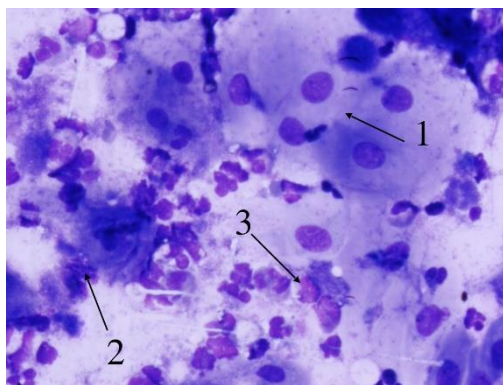


Figure 3. Photo dab-imprint from the surface of the oral mucosa in acute non-specific inflammation, the stage of exacerbation: 1 - intact single epithelial cells, 2 - the entire field is covered with the remnants of cellular elements, 3 - a significant number of white blood cells located in groups.

Epithelial cells of oral mucosa also change if the inflammation is associated with a viral infection. The cytological picture in this case will be characterised by presence of giant multi-nucleated cells on the place of erosions. This cells have flat-angulaited or changed shape with an increased number of nuclei. The number of nuclei can vary from 2 or more nuclei in such cells in Fig. 4.

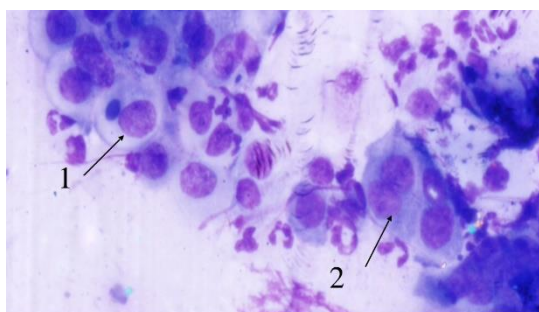


Figure 4. Photo of dab-imprint from the surface of erosion during inflammation of the oral mucosa of viral etiology: 1 - cells of the prickle layer of epithelium (small in size with a large nucleus), 2 - giant flat-angulaited binucleated cell.

Cytological changes in the oral mucosa during viral infections are detected only in the first two days after the appearance of erosive elements in the oral cavity. After some time, pathological multi-nucleated cells are not detected in cytological preparations due to morphological changes in the erosive surface.

It should be noted that the detection of multi-nucleated cells proves the viral nature of the disease. The method of cytological diagnosis should be carried out among patients with suspected viral nature of the disease of the oral mucosa, as well as in cases of atypical course of the disease.

Molecular biological method (PCR and DNA-hybridisation) is used to identify the type and kind of viruses and its amount in the biomaterial. The sensitivity and specificity of polymerase chain reaction (PCR) is 98% - 94%. PCR should be performed in cases of severe viral mixed infections. PCR results can be obtained in 3-6 hours. Oral fluid (saliva) or blood are materials for this study.

The cytological method is also used for the differential diagnosis of diseases that are accompanied by the formation of blisters of the oral mucosa. Acantholytic pemphigus is one of these diseases. Development of blisters is associated with the acantholysis of epithelial cells in Fig. 5.

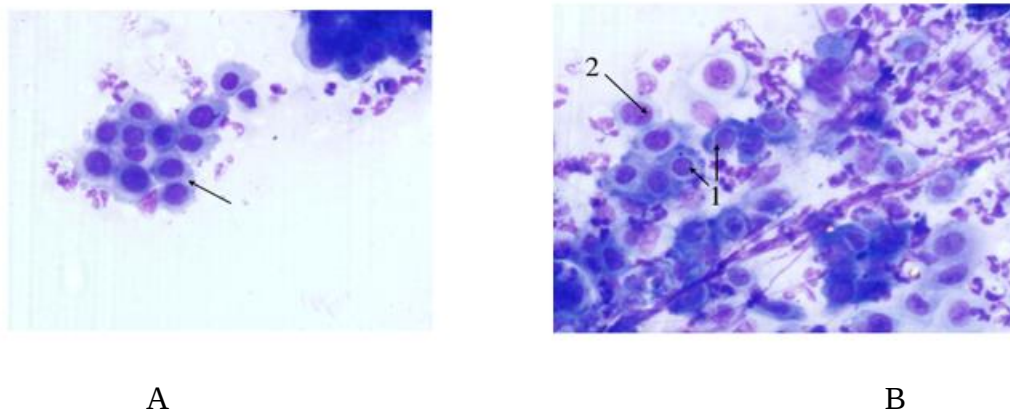


Figure 5. Photo of dab-imprint from the erosive surface of oral mucosa with acantholytic pemphigus. A - cells of the prickly layer of the epithelium. B-1– layers of Tzanck cells. B-2- progenitor Tzanck cell (cell from which Tzanck cells are formed).

The dab-imprints are recommended to be applied from an erosive surface in place of the blister. The ill-defined cells of the prickly layer of the epithelium are defined in the dab-imprint. This cells has a smaller size. These cells contain a large nucleus, which occupies almost the entire cell. The colour of such cells is

heterogeneous. The layers of ill-defined prickly cells of the epithelium are diagnosed in patients with acantholytic pemphigus in Fig. 5 - A.

The presence of Tzanck cells in dab-imprint is necessary for the diagnosis of acantholytic pemphigus. Tzanck cells are typical cells of this disease. They have a pronounced bright corolla around the enlarged nucleus. This corolla is a marker of Tzanck cells. Acantholytic Tzanck cells can be present in the preparations both in the form of single cells, and in a large number of cells located in layers in the visual field in Fig. 5. B-1.

Often, progenitor cells of Tzanck can be found in the preparations. These cells have a reduced size and a large nucleus (Fig. 5. B-2). The cytological examination is recommended to repeat. The final diagnosis of acantholytic pemphigus can be installed only after detection Tzanck cells in dab-imprint from the surface of erosion of the oral mucosa.

Young epithelial cells, juvenile cells (white blood cells), mononuclears, motile phagocytes are determined in the oral mucosa at the initial stage of erosion regeneration. There are also epithelial cells with a complete morphological structure (late cell) and white blood cells that actively phagocytise. The number of microorganisms is reduced.

There is a predominance of the number of cellular elements due to young epithelial cells over the number of morphologically mature white blood cells, mononuclear cells during the active regeneration of erosion.

The effectiveness of epithelialisation of erosion during the course of treatment can be determined by calculating the index of the cell-epithelial ratio Kimele E.V. :

$$IRCE = V_c / V_e,$$

where, V_k – the percentage of all cellular elements of the cytogram (lymphocyte cells, phagocytes, macrophages); V_e – the percentage of epithelial cells.

This index of intact oral mucosa is equal to 0.08 [117].

So, the cytological method is one of the most informative laboratory methods in the differential diagnosis of erosive and inflammatory lesions of the oral mucosa. This study is used to identify typical cellular changes of oral mucosa in diseases of various

etiology, including viral origins. The cytological method allows differential diagnosis of diseases of the oral mucosa in accordance with the principles of evidence-based medicine. This research method is also important for studying the effectiveness of the prescribed treatment by cytological monitoring of the process of epithelialisation of the oral mucosa.