ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

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აღწერა სტატიის გრაფიკული სექციებიდან სხვადასხვა სწორედ შესაძლო სტატია: 
IMPROVEMENT OF THE METHODOLOGY OF BIOMATERIAL COLLECTION FOR THE DIAGNOSIS OF THE ORAL CAVITY MUCOSA DISEASES


Poltava State Medical University, Poltava, Ukraine.

Abstract.

Aim: To improve the methodology for collecting material from lesions of the oral mucosa for exfoliative cytological examination.

Material and methods: A group of patients diagnosed with B37.0 Candida stomatitis was examined. To clarify the diagnosis, various methods of collecting biological material from the tongue of patients were used, namely, the method using a cytobrush with subsequent fixation of cytological material on a slide. The microbiota of the back of the tongue was analyzed in 12 patients with glossitis and 12 healthy subjects (the control group). The microscopic method of research was used - using an immersion microscope MICROMed@XS-3330, and the morphological and tinctorial properties of microorganisms were determined. In ten fields of view, the number of leukocytes, the nature of epithelial cells, and the presence of various microorganisms were detected and counted. A comparison of the quality of the use of the microscope method for the study of the tongue microbiota of patients with candidal glossitis was performed under the conditions of taking pathological material using a dental scalpel and an oral cytobrush. For a reasonable interpretation of the results and determination of their significance, a statistical analysis was performed to determine the frequency of detection of microorganisms in patients with glossitis and healthy subjects, depending on the nature of the material taken from the back of the tongue using a dental scalpel or cytobrush.

Results: The studies showed that the etiologic structure of glossitis pathogens was dominated by Candida yeast-like fungi, but cases of leptotrichosis aetiology were observed (16.7%). Monococci and gram-negative monobacteria were detected in all studied groups. An increase in the diversity of microorganisms was found when the material was taken with a cytobrush. The microbiota of all subjects differed depending on the type of instrument used for sampling. Thus, in the group of healthy individuals, the interdental brush helped to detect twice as many streptococci as a scalpel. In patients with candidiasis, a brush biopsy showed a 2.7-fold increase in gram-positive diplococci, twice as many streptococci and gram-positive bacilli, three times as many staphylococci, 2.25 times as many clusterforming gram-negative cocci, and 2.3 times as many gram-negative diplococci.

Conclusions: A significant increase in the diversity of microorganisms was observed with the cytobrush compared to the use of a dental scalpel. In patients with glossitis, the accumulation of keratinized epithelial cells was significantly higher compared to the presence of young cells in healthy subjects, regardless of the method of sampling.

Key words. Cytobrush, microbiota, candidiasis, oral mucosa, glossitis.

Introduction.

Diagnostics of diseases of the oral mucosa is always a complex and urgent problem in the practice of a dentist. Examination of the oral cavity is traditionally considered to be the necessary and best approach to detecting pathologies of the oral mucosa. It is a non-invasive method that can be quickly performed by many healthcare professionals without any additional cost to the patient. However, the evidence for visual inspection as an effective screening method remains controversial [1]. Experts have concluded that there is currently insufficient evidence to support or refute the use of oral examination as an effective screening test [2]. For the diagnosis of many inflammatory diseases of the oral mucosa, precancerous and oncological pathologies, blistering, and fungal lesions, exfoliative cytology is used for additional research methods [3,4]. It is considered an important method of morphological diagnosis, which allows assessment of the nature of the pathological process, changes in tissue reactivity and disease dynamics.

Aim: Our work was aimed to improve the methodology for collecting material from lesions of the oral mucosa for exfoliative cytological examination.

Materials and Methods.

Recognizing the main limitations of oral exfoliative cytology and the desire to improve the quality of smears and the sampling procedure by modifying the sampling tools to reduce the number of false-negative results, we proposed the use of a device for obtaining material from the surface of the tongue. The device is based on an interdental brush made of high-quality hypoallergenic materials: the villi are made of nylon, and the rod is made of a medical alloy that does not undergo oxidation. The device consists of two elements: a holder handle, which can be long or short; and a working part containing villi. The neck can be easily bent in either direction for comfort when taking material, and the nylon villi penetrate anatomical structures and can capture more cells from the lesion. This design of the interdental brush made it possible to make a device in the form of an oval brush on a rigid base, which increased the quality and area of sampling during exfoliative cytology (Figure 1).

This cytobrush also proved to be less traumatic for patients than scalps. Currently, there are no similar patented systems for oral exfoliative cytology in Ukraine. We examined a group of patients diagnosed with B37.0 Candida stomatitis (ICD). To clarify the diagnosis, we used different methods of collecting biological material from the back of the tongue of patients: using a dental scalpel, which is
Bacteria were present in small numbers, as well as single gram-positive streptococci and gram-negative filamentous negative cocci, and many gram-negative rod-shaped bacteria. Layers, a large number of gram-positive monococci and gram-negative bacilli. When a cytobrush was used on the same subject, the microscopic picture was generally consistent, but we also saw gram-positive yeast-like fungi of the genus Candida. Similarly, in the examination of a patient with candidal glossitis, when the material was taken with a scalpel in ten fields of view, we found epithelial cells of varying degrees of keratinization, many polymorphonuclear neutrophils, large numbers of gram-negative rod-shaped bacteria, many adherent gram-positive monococci on the epithelium, gram-positive lanceolate diplococci, and gram-negative filamentous bacteria. With the help of a dental brush biopsy, the smear showed mainly layers of keratinized epithelium, pseudocolocytes, and the presence of more than 10 leukocytes in each field of view. In addition to the above microorganisms, clusters of gram-negative cocci, gram-positive yeast-like fungi, and gram-negative spiral bacteria were additionally observed. In patients with leptotrichosis, the microscopic picture did not differ depending on the type of instrument used for sampling collection and was represented by layers of keratinized epithelium, mainly gram-negative bacillus-like bacteria, gram-positive monococci and gram-negative filamentous bacteria in large numbers.

Table 1 shows the data on the frequency of detection of microorganisms in patients with glossitis and healthy subjects, depending on the method of taking material from the back of the tongue using a dental scalpel or cytobrush (interdental brush biopsy).

<table>
<thead>
<tr>
<th>Microorganisms/groups to be tested</th>
<th>Control, scalpel</th>
<th>Control, cytobrush</th>
<th>Glossitis, scalpel</th>
<th>Glossitis, cytobrush</th>
</tr>
</thead>
<tbody>
<tr>
<td>gram-positive monococci</td>
<td>12 (100)</td>
<td>12 (100)</td>
<td>12 (100)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>gram-positive diplococci</td>
<td>4 (33,3)</td>
<td>7 (58,3)</td>
<td>3 (25)</td>
<td>8 (66,7)</td>
</tr>
<tr>
<td>gram-positive streptococci</td>
<td>4 (33,3)</td>
<td>8 (66,7)</td>
<td>2 (16,7)</td>
<td>4 (33,3)</td>
</tr>
<tr>
<td>gram-positive staphylococci</td>
<td>2 (16,7)</td>
<td>3 (25)</td>
<td>1 (8,3)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>gram-positive bacilli</td>
<td>4 (33,3)</td>
<td>5 (41,6)</td>
<td>1 (8,3)</td>
<td>2 (16,7)</td>
</tr>
<tr>
<td>gram-positive branched filamentous bacteria</td>
<td>2 (16,7)</td>
<td>3 (25)</td>
<td>3 (25)</td>
<td>5 (41,6)</td>
</tr>
<tr>
<td>gram-negative coccis</td>
<td>5 (41,6)</td>
<td>7 (58,3)</td>
<td>4 (33,3)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>gram-negative diplococci</td>
<td>1 (8,3)</td>
<td>2 (16,7)</td>
<td>3 (25)</td>
<td>7 (58,3)</td>
</tr>
<tr>
<td>gram-negative rod-shaped bacteria</td>
<td>12 (100)</td>
<td>12 (100)</td>
<td>12 (100)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>gram-negative filamentous bacteria</td>
<td>7 (58,3)</td>
<td>5 (41,6)</td>
<td>6 (50)</td>
<td>8 (66,7)</td>
</tr>
<tr>
<td>gram-negative spiral bacteria</td>
<td>1 (8,3)</td>
<td>2 (16,7)</td>
<td>2 (16,7)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>gram-positive yeast-like fungi</td>
<td>4 (33,3)</td>
<td>5 (41,6)</td>
<td>10 (83,3)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>young epithelium</td>
<td>3 (25)</td>
<td>5 (41,6)</td>
<td>1 (8,3)</td>
<td>1 (8,3)</td>
</tr>
<tr>
<td>keratinized epithelium</td>
<td>3 (25)</td>
<td>2 (16,7)</td>
<td>7 (58,3)</td>
<td>8 (66,7)</td>
</tr>
<tr>
<td>epithelium of varying degrees of keratinization</td>
<td>6 (50)</td>
<td>5 (41,6)</td>
<td>4 (33,3)</td>
<td>3 (25)</td>
</tr>
</tbody>
</table>
The analysis showed that a large number of gram-positive monococci and gram-negative rod-shaped bacteria were observed in the microbiota of all subjects. In patients with glossitis, in addition to the above microorganisms, yeast-like fungi were also predominantly detected.

The microbiota of all subjects differed depending on the type of instrument used for sampling. Thus, in a group of healthy individuals, an interdental brush helped to detect twice as many streptococci as a scalpel. In patients with candidiasis, a brush biopsy showed a 2.7-fold increase in gram-positive diplococci, twice as many gram-positive streptococci gram-positive bacilli, three times as many gram-positive staphylococci, 2.25 times as many gram-negative cluster-shaped cocci, and 2.3 times as many gram-negative diplococci.

Thus, both in a group of healthy individuals and in patients with candidal glossitis, the microbiota of the back of the tongue differs significantly depending on the type of instrument used to take pathological material. Thus, a significant increase in the diversity of microorganisms was observed with the use of an interdental brush compared to the use of a dental scalpel.

Regarding cytological studies, it can be stated that in patients with glossitis, the accumulation of keratinized epithelial cells significantly prevailed compared to the presence of young cells in healthy subjects, regardless of the biopsy method.

The studies showed that the etiologic structure of glossitis pathogens was dominated by gram-positive yeast-like fungi of the genus Candida, but cases of leptotrichosis aetiology were observed (16.7%). Gram-positive monococci and gram-negative monobacteria were detected in individuals of all groups. An increase in the diversity of microorganisms was found when the material was taken with a cytobrush.

Discussion.

Candida albicans is the main causative agent of fungal diseases in humans and can often exist as a commensal in the oral cavity, intestines, or genital tract of most people, controlled by the local microbiota, epithelial barriers, and immune defence.

Changes in the control system or functional impairment of these species-specific defences can lead to excessive fungal growth and the development of mucosal infections, particularly of the oropharynx, and patients with severe immunodeficiency may be susceptible to life-threatening systemic fungal infections [7].

In recent years, scientists have increasingly focused their attention on the critical importance of the microbiota in maintaining human immunity and protecting against many dental diseases, including candidal glossitis [6]. Studies show that the vast majority of cases of candidiasis are associated with serious microbiota disorders. Irrational antibiotic therapy plays a particularly important role in the spread of fungal infections, leading to the suppression of autochthonous bacteria, which are the main competitors of fungi in humans. They prevent yeast-like fungi from attaching to mucosal cell receptors, compete with them for food sources, block the penetration of Candida fungi through the mucosal barrier, inhibit their growth through the synthesis of antifungal compounds, and enhance the body's antifungal immune response.

When the microbiota is in a normal state, the immune system is fully functional, the full range of protective functions of resident microorganisms is realized, and fungi have little chance of increasing their populations and manifesting pathogenic properties. The risk of developing fungal infections increases dramatically in a pathological change of the composition and functions of the microbiota. Dysbiosis leads to significant immune disorders. Under conditions of dysbiosis, fungi of the Candida genus, without meeting resistance from the immune system and resident bacteria, can significantly increase their presence in the body and affect the mucous membranes of the oral cavity, oesophagus, gastrointestinal tract, and genitourinary system.

Oropharyngeal candidiasis is one of the most common manifestations of local dysbiosis and can occur in acute or chronic form. The predisposing factors for the development of glossitis include nutritional deficiencies, salivary hypofunction, smoking, wearing dentures, and T-cell immunity dysfunction, often in the elderly. Many acute cases are caused by treatment with broad-spectrum antibiotics [4,8]. The importance of the interaction between yeast-like fungi and the host microbiota in the transition from C. albicans commensalism to pathogenicity and the various factors that influence the composition of the oral microbiota and fungal colonization, and antifungal immunity are widely recognized. The study of the tongue microbiota of patients with candidal glossitis will provide opportunities for targeted complex therapy, including the use of not only antifungal antibiotics, but also probiotics, immunomodulators, antisepsics, and herbal remedies [9-11].

A significant addition to the study of the microbiota in the normal state and the development of infectious pathology of the oral cavity has been made using the latest methodical and methodological approaches, in particular, using genome sequencing and metaproteomics. However, to solve such a problem as improving the methodology for collecting material from lesions of the oral mucosa for exfoliative cytological examination, our data obtained by microscopic examination, firstly, correspond to the literature, and secondly, are sufficient to compare the results of using different tools for taking material [5,8,12].

The appearance and proportion of epithelial and other cells depend on the anatomical site of the oral cytology specimen. Atypical changes in epithelial cells are observed in ulcers and nonspecific inflammation due to irritation. Inflammation of the oral mucosa can be acute or chronic, infectious, or non-infectious in origin. Clinically, it manifests itself in the form of ulceration or erosion, which, in turn, are diffuse or localized, single or multiple [12]. As a result of inflammation, there is an increased rate of epithelial cell desquamation, so much so that the superficial and intermediate epithelial layers are partially or even completely replaced by deeper parabasal squamous cells. The background of an infectious smear from the oral cavity can be protein or hemorrhagic with the presence of polymorphonuclear leukocytes, eosinophils, mast cells, macrophages, lymphocytes, and plasma cells, depending on the type and cause of the infection, as well as the nutritional and immune status of the person. In chronic inflammatory processes, moderate phagocytosis is detected, and in acute inflammatory processes, active phagocytosis is detected, the number of keratinized cells and the keratinization index increase, cellular
and nuclear polymorphism are present, atypical forms of division are detected, and the number of mitoses is increased [12].

In dental practice, certain difficulties in the use of traditional diagnostic methods have been identified, which are associated with the peculiarities of the anatomical structure of various parts of the oral mucosa. The first researchers recognized the main limitations of oral exfoliative cytology and sought to improve the quality of smears. Thus, in gynaecology, after using a cytobrush in the diagnosis of dysplastic lesions of the cervix, the introduction of softer endocervical brushes significantly improved the ability to collect epithelial cells. However, in dental practice, recognizing the main limitations of oral exfoliative cytology and the desire to improve the quality of smears, trying to reduce the number of false-negative results, we proposed the use of an economical device for obtaining material from lesions, simplifying the procedure for taking samples by modifying the instruments for diagnosis. Thus, the use of the proposed cytobrush is an affordable, easy-to-use, economical method of taking material from the back of the tongue for obtaining a reliable and quick answer when studying the oral microbiota in the diagnosis of glossitis.

Conclusion.

Thus, in both groups of healthy individuals and patients with candidal glossitis, the microbiota of the back of the tongue differs significantly depending on the type of instrument used to take pathological material. Thus, a significant increase in the diversity of microorganisms was observed with the use of a cytobrush compared to a dental scalpel.

Regarding cytologic studies, it can be stated that in patients with glossitis, the accumulation of keratinized epithelial cells significantly prevailed compared to the presence of young cells in healthy subjects, regardless of the method of sampling.

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Conflict of interest.

The authors declare no conflict of interest regarding this article. The authors declare that this work does not create any conflict of interest for any person or institution.

Compliance with Ethics Requirements.

The authors declare that all procedures of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008.

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