

## THE INFLUENCE OF THE METHOD OF MATERIAL COLLECTION ON THE RESULTS OF CYTOMORPHOLOGICAL EXAMINATION IN THE DIAGNOSIS OF NEOPLASMS OF THE ORAL MUCOSA

*Chirag Darji*

*Assistant of Oncology department Samarkand state medical university*

*Khujakulov Sh.Sh.*

*Magistr of oncology department Samarkand state medical university*

*Dusmurodova Sh.Sh.*

*Student of medical laboratory department Samarkand state medical university*

**Abstract:** The oral mucosa and underlying tissues have a complex anatomical structure, which determines the specificity of the clinical course and treatment of tumors developing here. Malignant tumors that occur in the oral cavity, in most cases, have a structure of squamous cell keratinizing cancer. In a dental clinic, diagnostics of precancerous and tumor changes presents serious difficulties, the main reason for which is the low oncological alertness of dentists, as well as the lack of the necessary equipment and modern minimally invasive methods for early detection of cancer. Histological examination is rarely used in dentistry, since it involves the intravital collection of pathological tissue (biopsy) and has a number of disadvantages associated with the work with a sharp instrument, the possibility of bleeding, errors in collecting material, and the duration of waiting for the result from the pathologist. Considering the external localization of neoplasms of the ORAL MUCOSA and KCG, the procedure for collecting material for cytological examination is simple and highly sensitive (the sensitivity of the method is 90-95%).

**Aim:** assessment of the possibility of cytological diagnostics in the practical activities of a dentist and the influence of various methods of collecting material on the results of morphological analysis.

**Materials and methods of research.** A total of 114 samples from 38 patients who sought dental care with complaints of neoplasms in the oral cavity were analyzed. Material from each patient was collected in three ways with subsequent cytological examination.

**Results.** The obtained results indicate that the lowest percentage of detection of malignant changes in the cells of patients was observed with self-washing from the oral mucosae, and the highest percentage of pathologically altered cells was detected when collecting material using the smear print method. The obtained results of the cytological study were compared with the histological data, where various percentage matches were revealed depending on the method of collecting the material.

**Conclusion:** the use of the cytological method of research in dental practice with adequate material collection allows to identify malignant changes in cells in more than 80% of cases.

**Keywords:** cytological examination, neoplasms of the oral mucosa, preanalytical stage of cytological examination.

**Introduction:** The oral mucosa and underlying tissues have a complex anatomical structure, which determines the specificity of the clinical course and treatment of tumors developing here. Malignant tumors that arise in the oral cavity, in most cases (90-95%) have the structure of squamous cell keratinizing cancer. Among malignant tumors of the head and neck region, oral cancer is the second most common after laryngeal cancer [6]. Precancerous diseases of the oral mucosa (OM) and the red border of the lips (RBL) are an important problem of modern oncology and dentistry. A distinction is made between precancers with a high (obligatory) and low (optional) frequency of malignancy. According to D. Lascaris (2006), the probability of malignancy of verrucous leukoplakia is 20-40%, erosive 20-30%, flat 4.2%, lichen planus 1%. Abrasive precancerous cheilitis of Manganotti is classified as an obligate precancer with a high potential for malignancy in 9-42% of cases, Bowen's disease in 100% transforms into cancer (Mashkilleyson A.L., 1970). Chronic cracks, ulcers of Oral mucosa and KKG are also classified as optional precancers (Mashkilleyson, 1977) [1-4]. The occurrence of precancerous processes of Oral mucosa is facilitated by chronically acting factors of exogenous and endogenous nature. Their long-term exposure leads to the development of chronic inflammation, changes in metabolic processes, disruption of proliferative activity and keratinization processes in the epithelium of Oral mucosa and KKG with the formation of cracks, erosions and ulcers. This picture is aggravated by a decrease in the general reactivity of the body, caused by the influence of various viruses. Thus, the Epstein-Barr virus and human papillomavirus are involved in the development of ORAL MUCOSA cancer [5, 7, 8, 10]. The relevance of early diagnostics, choice of treatment methods and tactics of management of dental patients with precancerous processes are closely related to the prevention of malignancy of the pathological process. In a dental clinic, diagnostics of precancerous and tumor changes presents serious difficulties. The main reason for untimely diagnostics is low oncological alertness of dentists due to insufficient awareness of the etiology and pathogenesis of precancerous diseases, signs of malignancy of neoplasms, methods of their diagnosis, as well as the lack of necessary equipment and modern minimally invasive methods of early detection of cancer. In this regard, prevention, early minimally invasive detection of neoplasms, timely treatment are an important task of modern medicine. Considering the external localization of neoplasms of Oral mucosa and KKG, the procedure for collecting material for cytological examination is simple. The most common cytological examination of the epithelium of Oral mucosa is in order to identify disturbances in the differentiation of epithelial cells against the background of various pathological processes. The sensitivity of cytological examination is 90-95% [3]. Histological examination is rarely used in dentistry, as it involves the intravital collection of pathological tissue (biopsy) and has a number of disadvantages associated with the use of a sharp instrument, the possibility of bleeding, errors in collecting material, and the length of time it takes to receive results from a pathologist. The aim of the study was to assess the possibility of cytological diagnostics in the practical activities of a dentist and the impact of various methods of collecting material on the results of morphological analysis.

**Materials and methods of research:** A total of 114 cytological preparations from 38 patients seeking dental care with complaints of ulcers and neoplasms in the oral cavity were analyzed. The material from each patient was collected in three ways:

- a smear-imprint from the affected area, obtained by applying a piece of rubber band to the pathological focus with its subsequent imprint on a glass slide;
- a scraping of the lesion, for which type "a" (universal) cytological brushes were used, which after collecting the material were placed in a test tube with a transport nutrient medium;

- an independent wash from the oral cavity, performed by the patient using 10.0 ml of sterile 5% glucose solution after cleaning the oral cavity with distilled water.
- In order to obtain adequate (reliable) results, the collection of material began with an independent wash from the oral cavity, after which smears-imprints and scrapings from the pathological focus were performed. After collection of the biomaterial, cytological preparations were prepared in the traditional way with Romanovsky staining. Visualization of cytological preparations was performed using a Zeiss Primo Star microscope (Carl Zeiss, Germany).

**Results and discussions:** The average age of patients in the study was  $57 \pm 3.4$  years, the male to female ratio was 2.17/1.0. The duration of the disease varied from 6 to 24 months. Cytological examination of patient material in the case of imprint smears showed the following picture: the conclusion of carcinoma was given to 16 patients, suspicion of cancer - 7, inflammatory process, including trichomonas elongate - 9, hyperkeratosis - 2, no pathology - 4 patients. When reviewing the material from Oral mucosa scrapings, the conclusion of the presence of carcinoma was given to 9 patients, suspicion of cancer - 3, hyperkeratosis - 1, inflammatory process, including trichomonas elongate - 5, pathology was not detected in 20 patients. The results of cytological examination of the material of independent swabs from the oral cavity were distributed as follows: carcinoma - 3, inflammation - 9, hyperkeratosis - 1, no pathology was detected - 25 patients (Table 1).

In all cases of carcinoma, the tissue type was defined as squamous cell carcinoma with varying degrees of pathological keratinization or without it. At the same time, in the cytological picture at the light-optical level, separately lying cells of the multilayered squamous epithelium (mPE) with pronounced signs of cellular and nuclear atypia, as well as the presence of pathological keratinization in the cytoplasm were observed (Fig. 1).

When microscopy of cytological preparations without pathology of the oral cavity, the cytological picture showed the presence of unchanged mPE cells, mainly of the superficial layer, background elements in the form of single neutrophilic leukocytes in the field of vision (Fig. 2).

The cytological picture of the inflammatory process observed in the studied preparations is characterized at the microscopic level by the presence of layers of mPE of various sizes, as well as separately lying epithelial cells, in part of the epithelial component signs of parakeratosis and reactive changes were noted, the background component is represented by an abundant number of degeneratively altered neutrophilic leukocytes and a variety of microflora (bacteria, elements of the Candida fungus, protozoa - Trichomonas elongate, as well as indirect signs of viral infection) (Fig. 3). Combinations of various variants of microflora and pathological processes in oral mucosa are presented in Table 2.

Comparison of the cytological analysis results with those within the histological examination was carried out in 37 of 38 patients (97.3%). In one patient, there was no possibility of conducting a histological examination within the outpatient link. The results of the cytological examination were compared with the histological ones separately depending on the method of collecting the material. As a result of the comparison, the following results were obtained:

- when taking a smear of the imprint from the affected area in 30 patients (in 81.1% of cases), no false positive/false negative results were noted;
- when scraping from the pathological focus in 22 patients (in 59.4% of cases), no false positive results were found, false negative - 8.
- when washing from the oral cavity in 9 patients (in 24.3% of cases), no false positive results were found, false negative - 13.

- It should be noted that in 6 patients (15.8%), the collection of cytological material was preceded by invasive intervention (biopsy) at the place of residence within 1 to 4 weeks. Examples of cytohistological conclusions are presented in Table 3.

**Table 1. Results of cytological examination of the oral mucosa material**

	Self flushing		Smear - imprint		Scraping	
	abs	%	abs	%	abs	%
Carcinoma	3	7,89	16	42,1	9	23,6
Suspicion of cancer	-	-	7	18,42	3	7,89
Inflammatory process including <i>Trichomonas elongata</i>	9	23,6	9	23,6	5	13,16
Hyperkeratosis	1	2,63	2	5,26	1	2,63
No pathology	25	65,88	4	10,5	20	52,6

**Table 2. Combination of oral microflora with pathological processes of oral mucosa**

	Carcinoma of the oral mucosa	Suspicion of cancer	Inflammation	Hyperkeratosis	No pathology
<i>T. elongate</i>	3	1	5	-	1
<i>Candida</i>	-	-	4	-	-
Viral infection (indirect signs)	4	2	1	-	3
Combination of <i>T. elongate</i> + viral infection	1	2	1	-	-

**Table 3. Results of comparison of cyto-histological diagnoses**

Cytological diagnosis	Histological diagnosis	Reason for the discrepancy
The preparation contains single epithelial cells with signs of atypia, related to squamous cell keratinizing carcinoma. <i>Trichomonas elongate</i> are noted.	Squamous cell carcinoma with a tendency to keratinization	low cellularity of cytological material
Cytological picture of therapeutic pathomorphosis	There is no tumor growth within the material.	Changes in individual cells at the cytological level are more clearly visible
The cytological picture does not allow to exclude the presence of PCR	Squamous cell nonkeratinizing carcinoma with ulceration	not sharply expressed cellular changes
against the background of purulent-necrotic masses, a small number of MPE cells with signs of atypia related to PCR are noted	Layers of necrosis with keratinizing cancer complexes	low cellularity of cytological material, predominance of background elements

Poor cellular composition: against the background of purulent-necrotic masses, a small number of MPE cells with signs of dyskaryosis and grade 2 dysplasia are noted	Squamous cell carcinoma with a tendency to keratinization	Predominance of background elements. Not pronounced cellular changes, collection of cytological material 2 weeks after biopsy
In the preparation of MPE cells, without atypia, a small number of cells with signs of metaplasia, single cells with signs of hyperkeratosis	Squamous cell carcinoma with a tendency to keratinization	Collection of cytological material 1.5 weeks after biopsy
The preparation contains purulent-necrotic masses, against the background of which there is a small number of MPE cells without atypia, and single cells with signs of hyperkeratosis are noted.	Verrucous leukoplakia with grade 1 dysplasia and adjacent structureless protein masses, impregnated with leukocytes. Possibly, the material was taken near the tumor growth	Predominance of background elements
Cytological features of carcinoma with basal cell differentiation	Squamous cell carcinoma with keratinization	
Cytogram of dysplasia grades 2-3	Well differentiated squamous cell carcinoma	not pronounced cellular changes
against the background of neutrophilic leukocytes and histiocytic cells, single MPE cells with signs of atypia related to PCr are noted	Well differentiated squamous cell carcinoma	Low number of epithelial cells in cytological material

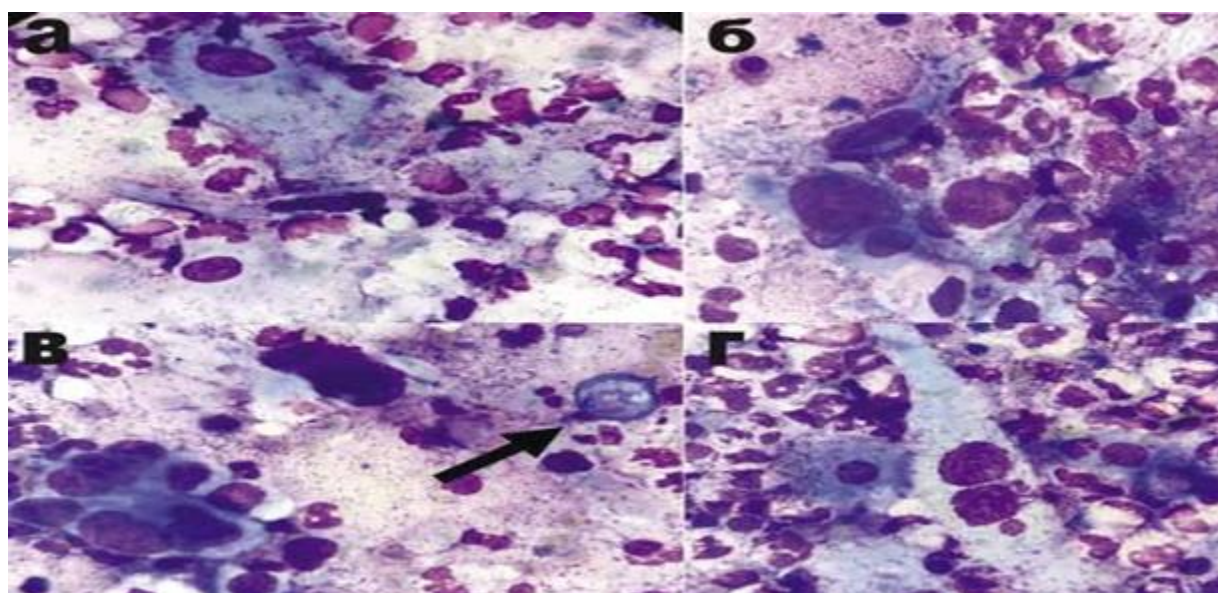


Fig. 1. Material from the oral mucosa. Cytological picture of squamous cell carcinoma with keratinization. Romanovsky staining, magnification x1000

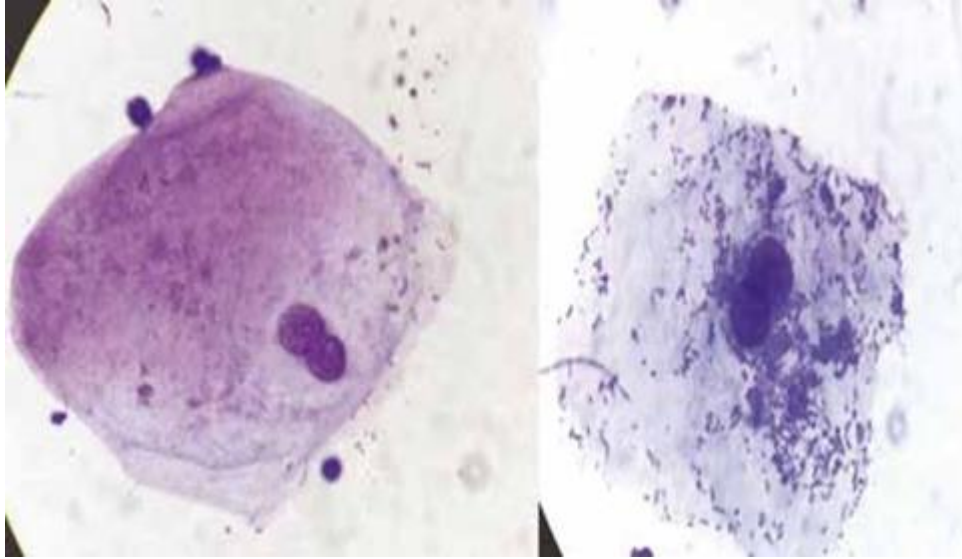


Fig. 2. Material from the oral mucosa. Cytological picture is unremarkable. Romanovsky staining, magnification x1000

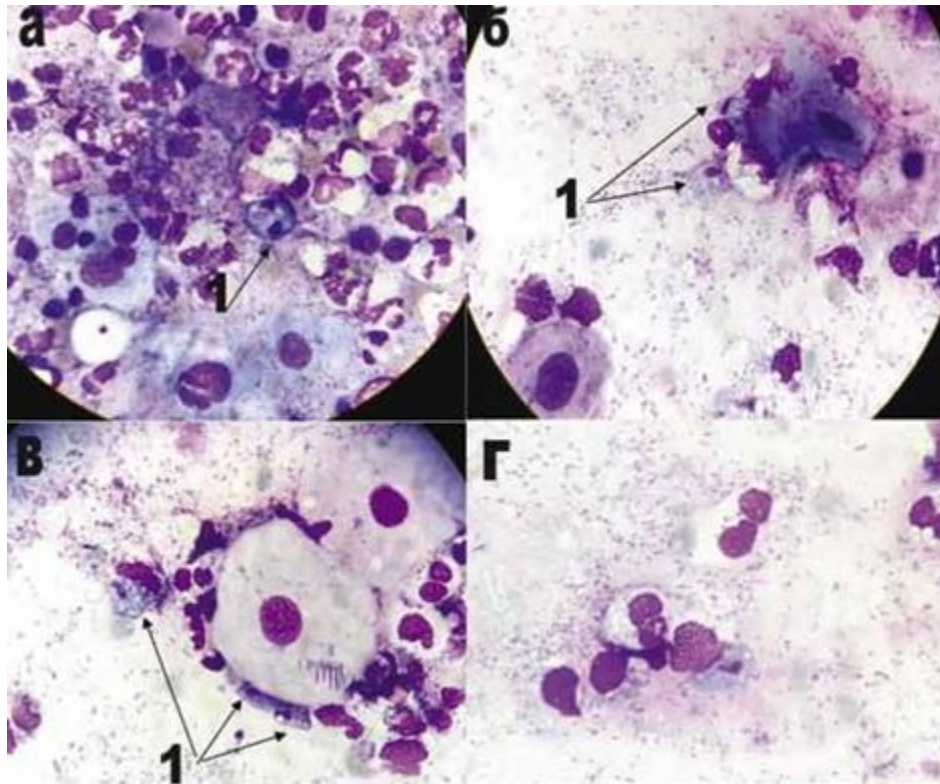


Fig. 3. Material from the oral mucosa. Cytological picture of the inflammatory process, including *Trichomonas elongata*. Romanovsky staining, magnification x1000

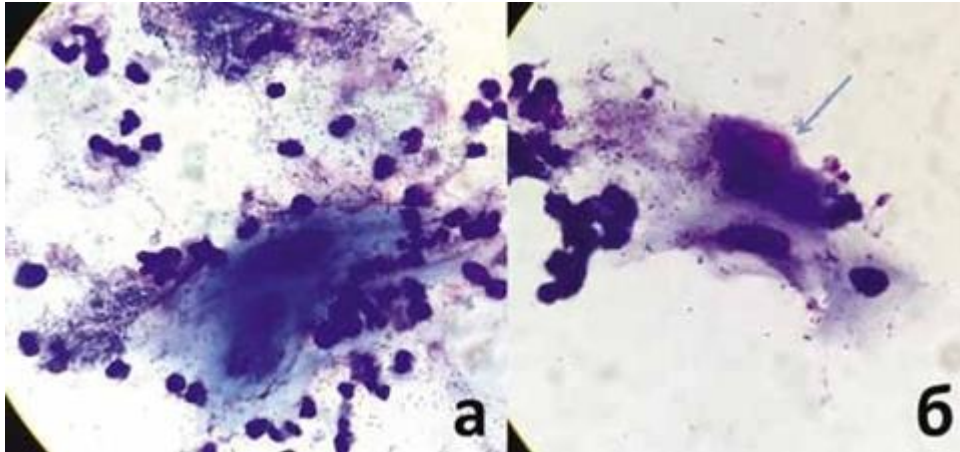


Fig. 4. Cytological preparation from the oral mucosa with a poor cellular composition. Romanovsky staining, magnification x1000

The analysis of the obtained results allowed us to establish that when collecting material by the imprint method from the pathological focus, the maximum concentration of pathologically altered cells and their relatively uniform distribution over the area of the preparation were detected in cytological preparations. It should be noted that in some cytological preparations, background elements prevailed over pathologically altered ones. In the samples obtained after collecting material by scraping from the lesion site, low cellularity was observed, but signs of malignancy of cells were clearly visible against the background of the absence of pathogenic microflora (Fig. 4). Analyzing the reasons for such a difference when comparing cytological and histological studies, several aspects are distinguished that can be divided into those common to all collection methods and specific ones encountered in each of the above-mentioned methods. The main and general reason for the discrepancy in the research results was the collection of material for cytological examination. Speaking about the reasons influencing the research results for each of the collection methods, the following can be highlighted:

- 1) during the smear of the imprint, the decrease in the quality of the cytological material is due to the difficult access to it during the collection process, as well as the lack of standardized tools that allow for a high-quality collection procedure (cytological brushes, spatulas, probes were used);
- 2) during scraping, the deterioration in the quality of the obtained material is associated with the adhesion of part of it on the surface of the cytological brushes and, accordingly, the impossibility of a comprehensive assessment;
- 3) the low quality of the material obtained as a result of self-washing from the oral cavity directly depends on the patient's violation of the step-by-step collection technology.

At the same time, the washout from oral mucosa is characterized by high cellularity of the resulting cytological preparations.

**Conclusion:** The use of the cytological method of examination in dental practice allows to detect malignant changes in cells in more than 80% of cases. The greatest efficiency of detection of pathological changes was noted when collecting material by the smear-imprint method directly from the site of the lesion, which is explained by the maximum concentration of pathologically changed cells and the exclusion of the patient's influence on the method of collecting material. Previous invasive intervention reduces the probability of detecting pathological cellular changes in cytological material by 15.8%.

## Literature

1. Banchenko G.V., Molochkov V.a., Kryajeva S.S., Balyun d.G. Leykoplakiya i blizkiye k ney porajeniya slizistoy obolochki polosti rta // rossiyskiy jurnal kojnyx i venericheskix bolezney. — 2001. — № 5. — S. 4-8.
2. Bloxin n.n., Peterson B.ye. Klinicheskaya onkologiya. T.— M, 1979.
3. Быков V.L. Gistologiya i embriologiya organov polosti rta cheloveka: Uchebnoye posobiye. izdaniye vtoroye, ispravlennoye. — SPb «Spesialnaya literatura», 1998. — 248 s.
4. Gileeva o.S., Libik T.V., Pozdnyakova a.a., Satyukova L.Ya. Predrakovyye zabolevaniya v strukture patologii slizistoy obolochki polosti rta (po dannym lecheno konsltativnogo priyema) // Problemy stomatologii. — 2012. — №2. — S. 3-9.
5. Gutkovskaya ye.a., Smolyakova r.M., Iukoves a.G., Franskevich T.V.. Papillomovirusy cheloveka i virus Epshteyna-Barr pri rake polosti rta, rotoglotki i gortani: geneticheskaya karakteristika i chastota vyavleniya // onkologicheskii jurnal. — 2012. — T. 6. — № 1 (21). — S. 71-74.
6. Kaprin a.d., Starinskiy V.V., Petrova G.V. Sostoyaniye onkologicheskoy pomoshchi naseleniyu rossii v 2017 godu. — M.: Mnioi im. P.a. Gersena-filial FGBU «nMiS radiologii» Minzdrava rossii, 2018. — ill. — 236 s.
7. Nistratov G.P., Svetitskiy P.V., Zыkova T.a. i dr. Vliyaniye virusov Epshteyna-Barr i papilomy cheloveka na techeniye raka organov polosti rta // Sovremennyye problemy nauki i obrazovaniya. — 2014. — № 6.
8. Pozdnyakova T.i., Volkov ye.a., Smirnova Yu.a. i dr. Tendentsii rasprostranennosti predrakovykh zabolevaniy slizistoy obolochki polosti rta // obrazovaniye, nauka i praktika v stomatologii po obyedinennoy tematike: Stomatologiya i sotsialno-znachimyye zabolevaniya: Sb. tr. 10-y Vseros. nauch.-prakt. konf. — SPb: «Chelovek», 2013. — S. 186-187.
9. Gansev Sh.X. G 19 onkologiya: Uchebnik dlya studentov meditsinskix vuzov. 2-ye izd., ispr. i dop. — M.: ooo «Meditsinskoye informatsionnoye agentstvo», 2006. — С. 38-54.
10. Ang K.K., Harris J., Wheeler R. et al. Human Papillomavirus and Survival of Patients with Oropharyngeal Cancer // New England Journal of Medicine. — 2010. — Vol. 363. — № 1. — P. 24-35.
11. I.A. Kruglova<sup>1</sup>, S.V. Zinoviev<sup>2</sup>, A.E. Pursanova<sup>2</sup>, O.V. Utkin<sup>3</sup>, A.N. Denisenko<sup>1</sup>