Cytological Analysis of Surface Epithelial Layers of Oral Mucosa

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Various methods of cytological analysis of surface epithelial layers of the buccal mucosa are compared in patients in need of dentures. Different informative value of cytological prints, scrapings, and washings off the oral cavity for analysis of exfoliated epitheliocytes is shown. The described methods help to evaluate the regeneration potentialities of the buccal mucosa epitheliaum in the course of treatment.

Key Words: *buccal mucosa; exfoliated epitheliocytes; cytological methods*

Studies of functional abnormalities in the whole organism and its systems require adequate informative methods of analysis [1,2,4]. Such studies help to detect and more accurately diagnose the disease, evaluate organism's reactions to environmental factors, and study the interactions between implants and adjacent tissues [3,5,7,8]. Studies of the buccal mucosa status are important for evaluating the status of the maxillodental system and the whole organism. Surface epithelium is the main functional part of the buccal mucosa which is the first to react to various environmental factors [1,2]. M. A. Yasinovskii [9] described a correlation between the state of the buccal mucosa the whole organism (effect of internal factors), in particular for mercury and lead poisoning, gastritis, and dyspepsia. It should be emphasize that studies of the morphology and function of surface epithelial layers are important for understanding the processes in the oral mucosa during orthodontic treatment [10-13].

Evaluation of the patient's status helps to choose the method and strategy of treatment in disorders of the morphology and function of the oral mucosa. Qualitative and quantitative cytological studies acquire special importance, particularly in evaluation of the regeneratory processes. We compared the informative value of different cytological methods in examination of the oral mucosa in patients in need of orthodontic treatment.

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MATERIALS AND METHODS

Complex cytological analysis of exfoliated epitheliocytes in imprints, scrapings, and washings from the oral cavity was carried out in patients to be fitted with dentures.

Imprints were obtained by 2-cm rubber bars (16 mm^2 cross section parallelepiped with rounded corners). Disposable bars were made from soft white rubber and autoclaved. For obtaining an impression, a sterile bar was taken with pincers, slightly pressed to a certain part of the mucosa, and the mucosal discharge was collected from protrusions and depressions, including the sites difficult for collection.

Two imprints were prepared from each site: one for studies of cell composition and an other for analysis of microflora (Gram's staining and disposition with respect to cell elements). The former was fixed for 15 min in 96° ethanol and stained according to Romanowsky—Giemsa for 20 min at 37°C and a other was dried in flame and Gram-stained. Imprints were examined under microscope at ×1350.

The method for obtaining cytological material by consecutive washings (rinses) was suggested by M. A. Yasinovskii [9]. Washing from the oral cavity was

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fixed as described elsewhere [6] for bronchoalveolar lavage. Oral rinsing was performed at the same time of the day after three preliminary rinsing of the oral cavity with water; the patients were to follow a certain daily routine. The oral cavity was rinsed for 1 min with 10 ml sterile normal saline by active movements of the lips and cheeks, after which the patient thoroughly split the water into a clean glass, the fluid was stirred with a glass stick, and 1 ml^3 was taken for cytosis evaluation in a Goryaev chamber.

After that fluid was centrifugated for 10 min at 600*g*. Supernatant was thoroughly removed and a smear was prepared on a slide. The smears were dried on air at ambient temperature (for about 15 min), and fixed for 20 min in 96° ethanol, and stained with Romanovskii—Giemsa stain.

Surface scraping was made with a 5-mm histological spatula. Scrapings were carefully collected (without scarification) from the palatal mucosa of alveolar process 1 cm centrally to the first molars. The material was homogenized on a slide in a drop of sterile normal saline. Due to this procedure the material was evenly disposed on the glass thus allowing identification of all cell elements in the scraping. After drying on air the preparations were fixed in 96° ethanol and stained routinely.

All preparations of imprints and smears from oral washings and scrapings were used for studies of cell composition. Preparations obtained by imprints and scrapings allowed identification of cells from strictly determined sites of the oral mucosa.

The studies were carried out using a Docuval universal biological microscope (K. Zeiss). Surface epitheliocytes with different states of the nuclei and without nuclei were seen on the preparations. Cells were evaluated and classified by keratinization [4,8]. Cells were counted under an optic microscope at ×1350. At least 200 cells were examined in each case [3]. The intensity of keratinization of multilayer squamous epithelium was evaluated using the keratinization index (I_K) [4,13] which is used in periodontology:

$$I_K = (\text{Anucleated cells/Total number of epithelial cells}) \times 100\%.$$

Cell formulae (cytograms) were determined for each patient.

RESULTS

The quality of the resultant imprints allowed identification of cell elements (Fig. 1), but the cells were unevenly distributed in the preparation, overlapped, convoluted, smashed, had wrinkles, which impeded evaluation of their status.

Smears made from the precipitate of oral fluid allowed identification of all types and states of cell elements. The cells were well spread on the slides, lay free, and overlapped only in cases of abundant mucosal discharge (Fig. 2). Leukocytes were evenly distributed in smears and were easily identified even in preparations with conglomerations.

Fig. 1. Imprint of the hard palate mucosa from a patient with denture intolerance. Anucleated epitheliocytes with numerous microorganisms on their surface predominate. Uneven distribution of cells in the preparation. Here and in Figs. 2, 3: Romanowsky—Giemsa staining, ×200.

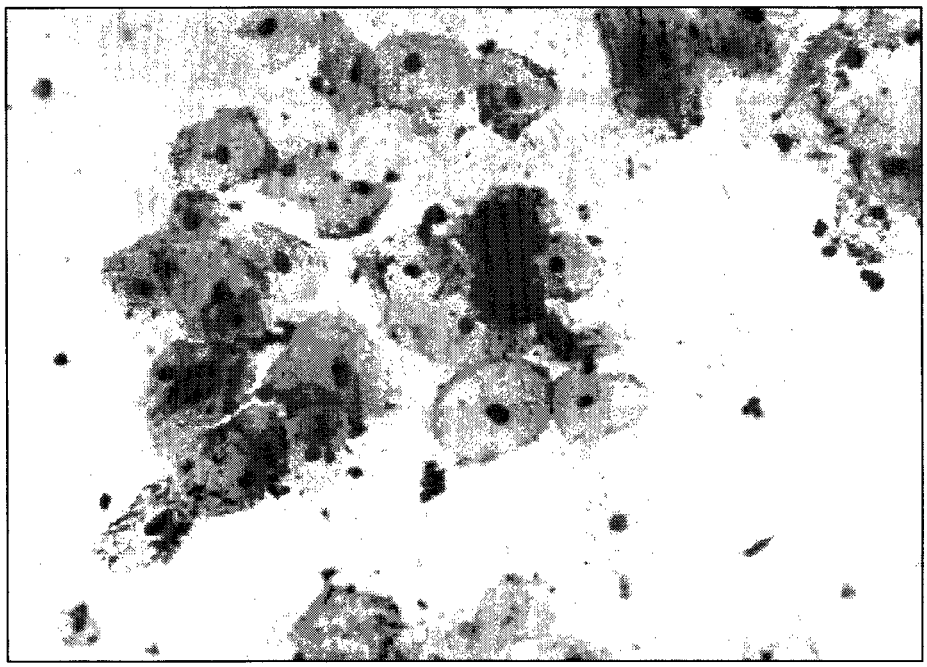

Fig. 2. Oral lavage before orthodontic treatment. Nucleated epitheliocytes predominate.

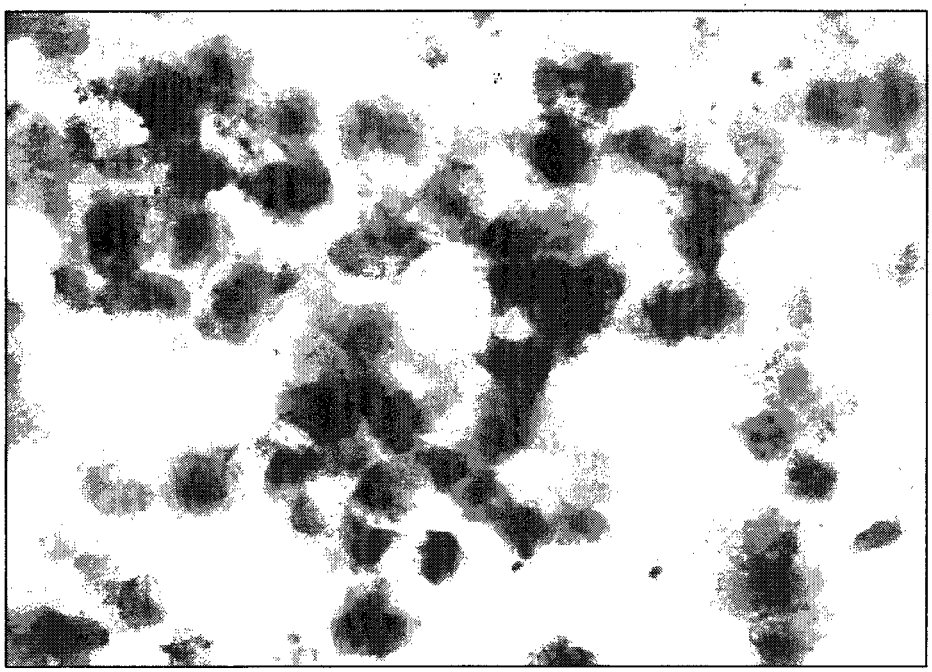

Fig. 3. Scraping from the hard palate mucosa. Anucleated epitheliocytes predominate.

Analysis of the cytological material obtained by lavage allowed evaluation of the oral mucosa status mainly by the leukocyte to epitheliocyte ratio. Lavage fluid included cells from the entire surface of the oral mucosa, which impeded differential evaluation [2].

Surface scraping with subsequent homogenization of the material most demonstrative characterized the examined sites of the oral cavity (Fig. 3). That is why we have chosen this method for studies of the oral mucosa, *e. g.*, for the analysis of adaptive reaction in denture bed to basic materials.

For studies of local reactions of the oral mucosa, methods adequate to the studied pathology and conditions of work with patients have to be chosen. In some cases the state and viability of large areas of the oral mucosa can be evaluated over time by analyzing oral discharge. This information is provided by cytological method and the material for this analysis can be collected by simple, available, and painless methods, such as smear imprints, lavage, and scraping.

Therefore, apart from clinical observations, objective evaluation of the state and regeneratory potential of the oral mucosa are needed for understanding the

processes in the oral cavity accompanying different pathological conditions and orthodontic treatment.

Comparison of our results of cytological study of exfoliated epithelial cells performed by the methods of imprints, scraping and washing from the oral mucosa with the published reports [1,4,13] showed that target samples are most informative for evaluation of keratinization of the epithelium. Surface scraping with subsequent homogenization of the material provides more information about processes in the buccal mucosa epithelium the.

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