ORIGINAL ARTICLE

MORPHOLOGICAL CHARACTERISTICS OF DIABETIC GLOSSITIS

DOI: 10.36740/WLek202203109

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ABSTRACT

The aim: To identify characteristic features of structural change of the dorsal part of the mucous membrane of the tongue (MMT) in experimental streptozotocin-induced diabetes (ESID).

Materials and methods: The study included 20 adult white male rats of Vistar line (body weight 180-200 g), which were equally divided into 2 groups: experimental (simulated streptozotocin diabetes mellitus) and control ones

Results: 8 weeks after the beginning of ESID modeling, the changes in MMT are particularly pronounced. A large number of lamellar structures and keratin conglomerates are found on the surface of MMT. This phenomenon is closely correlated (r=0.70) with a decrease in the absorption capacity of superficial epitheliocytes and an increase in the number of heterogeneous microflora on the impression smear with low activity of leukocyte elements. The number of epitheliocytes of differentiation stages I-III continues to increase, and the number of epitheliocytes of differentiation stages IV-VI diminishes, which leads to a significant decrease in the index of cell differentiation and an increase in the nuclear-cytoplasmic ratio. Such changes in MMT impression smears indicate active processes of epithelial desquamation with increasing duration of ESID.

Conclusions: Thus, the morphological changes of MMT in ESID are characterized by a diverse combination of atrophic and hyperplastic processes, resulting in uneven thickening of multilayered squamous epithelium. There are pronounced dystrophic changes in the epitheliocytes of the stratum corneum (dyskeratosis, parakeratosis) in the area of the taste buds. All areas of MMT are inflamed which indicates the development of diabetic glossitis.

KEY WORDS: tongue, mucosa, microcirculatory flow, streptozotocin-induced diabetes

Wiad Lek. 2022;75(3):619-623

INTRODUCTION

The mucous membrane of the tongue (MMT) is one of the important structures of the body. It performs multifaceted functions throughout life. Clinical changes of MMT are determined by morphofunctional features and caused by its localization in the first division of the digestive system. Due to the general neuroreflex regulation, chronic diseases, in particular diabetes mellitus (DM), have a special effect on the tissues of the tongue [1, 2]. The neurotrophic component in diabetes plays an important role in pathogenesis of destructive and inflammatory lesions of the tongue, but the mechanism of development of these lesions and morphological manifestations have not been studied enough. The issue of the state of the tongue in diabetes has been covered repeatedly in the scientific literature, but researchers described only the clinical manifestations of diabetic glossitis (DG) [3 4], as well as some features of laboratory diagnosis [5].

THE AIM

The aim of the study was to identify characteristic features of the structural change of the dorsal part of the mucous membrane of the tongue (MMT) in experimental streptozotocin-induced diabetes (ESID).

MATERIALS AND METHODS

The study included 20 adult white male rats of Vistar line (body weight 180-200 g), which were equally divided into 2 groups: experimental and control ones. The experimental group (10 rats) received intraperitoneally streptozotocin "SIGMA" (USA) which was diluted in 0.1 M citrate buffer with a pH of 4.5 (at the rate of 6 mg per 100 g of body weight), (Patent of Ukraine No. 62966; 11.02.2011, published on 20.09.2011, Bulletin No. 18). The control group (10 rats) in an equivalent dose received intraperitoneally 0.1 M citrate buffer with a pH of 4.5. The development of ESID was monitored by blood glucose levels, which were measured daily on an empty stomach in a drop of blood from the tail vein on an Assu-Chec Active (Germany) glucometer. The material was collected 8 weeks after the start of ESID modelling, after previous euthanasia, under thiopental anesthesia.

Animal experiments were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), the Law of Ukraine from December 15, 2009 and orders of the Ministry of Health of Ukraine No. 690 from 23.09.2009, No. 616 from 03.08.2012 (expert opinion of the commission on ethics

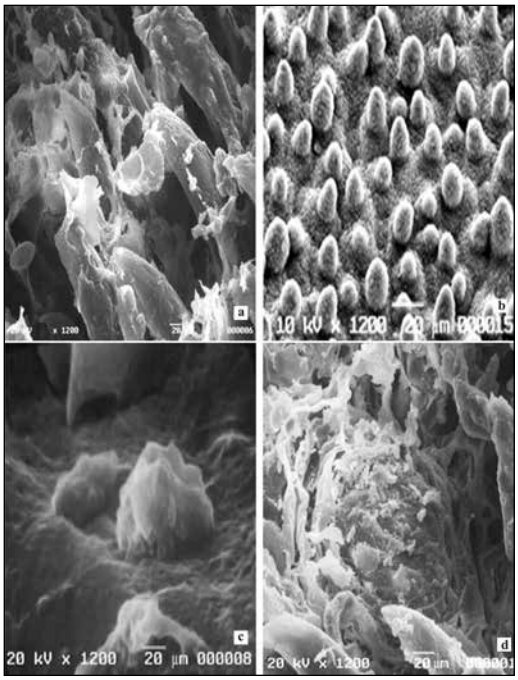

Fig. 1. Deposition of horny scales on the surface of filiform (a), cone-shaped (b), fungiform (c) and circumvallate (d) papillae of the mucous membrane of the tongue 8 weeks after the start of ESID modeling. Scanning electron microscopy. Electronic microphotographs. Magn.: x 1200.

of SHEI "Ivano-Frankivsk National Medical University", protocol No. 88/16 from 02.03 .2016).

For histological examination, pieces of the tongue were fixed in 10% neutral formalin, embedded in paraffin blocks; sections 5-7 μm thick were made with their following staining with hematoxylin and eosin.

To study the cytological characteristics of MMT, the impression smears on sterile slides were stained according to Romanowski-Gimza, viewed under a binocular light microscope MS 300 (THR) and photographed using a Digital camera for microscope DCM 900, installed in its tube with a resolution of 1200x1600 and saved in TIF format. The degree of destruction of epithelial cells was evaluated on the tissue specimen, the nuclear-cytoplasmic ratio (NCR) of epithelial cells was measured, on its basis the stages of differentiation of each epitheliocyte were evaluated. Then, the index of cell differentiation (ICD) was calculated by the formula ICD = 1a + 2b + 3c + 4d + 5d + 6e, where 1-6 is the numerical designation of the differentiation stages, a, b, c, d, e, e – the percentage of cells of the corresponding differentiation stage [6]. The keratinization index (KI) was determined by calculating the percentage of non-nuclear cells on a cytological preparation.

For transmission electron microscopic examination, pieces of the tongue were fixed in 2% osmium tetroxide solution, performed and contrasted according to the conventional method. The investigation of the material was performed using an electron microscope PEM-125 K, at an accelerating voltage of 75 kV, followed by photography. For scanning electron microscopy of the mucous membrane, the tongue was fixed in 10% neutral formalin, dehydrated in series of ethanol and acetone of increasing concentration. After that, it was dried by the critical point transition method. The samples were sprayed with carbon (at an angle of 90°), shaded with aluminum (at an angle of 15°) and an electrically conductive layer of silver was created (15 nm). The samples were viewed under the scanning electron microscope REMMA-102E ("SELMI", Ukraine) with an accelerating voltage of 10 and 20 kV.

Microphotographs were used for morphometric studies. Morphometry was performed using NIH USA "Image J" and "Bio Vision 4.01" programs in manual mode, taking into account magnifications. Computer data processing was performed using the statistical package Stat. Soft. Inc; Tulsa, OK, USA; Statistica 6. Statistical changes were considered significant when the level of statistical significance was $p<0.05$.

RESULTS

In the course of ESID, there is a gradual probable increase in the level of glucose and glycosylated hemoglobin, which 8 weeks after the beginning of the experiment, respectively, are: 18.6±0.36 mmol/l (control – 5.2±0.56 mmol/l, $p<0.05$) and 9.8±0.31% (control – 2.2±0.34%, $p<0.05$); it indicates the development of persistent decompensated diabetes. 8 weeks after the start of ESID modeling, the changes in MMT are particularly pronounced. A large number of lamellar structures and keratin conglomerates are found on the surface of MMT. These structures are distinctly seen on the filiform and circumvallate papillae. In some areas, the entire surface of the papillae is covered with small globular structures, which look like mulberry fruit (Fig. 1). This phenomenon is closely correlated ($r=0.70$) with a decrease in the absorption capacity of surface epitheliocytes and an increase in the number of heterogeneous microflora on the impression smear with low activity of leukocyte elements. Compared with the control group of animals, 8 weeks after the start of ESID modeling on preparations taken from the mucous membrane of the tongue, cell complexes were often observed. Their number exceeds 4-5 cells in each layer. Their edges have a sharp contour which is in some areas scalloped. The number of microorganisms is significantly increased and the number of lymphocytes is increased by 23.4% (20-30 in the field of view).

It should be noted that 8 weeks after the beginning of ESID modeling, a variegated cytological picture of MMT is observed, which is characterized by a combination of atrophic and hyperplastic processes. There is an increase in cells of the 1st and 2nd class of the destruction, respectively, to 30.7±2.23% (control – 10.8±2.02%, $p<0.01$) and 27.6±2.38% (control – 1.6±0.32%, $p<0.001$), with a decrease in cells from 0th class of destruction to 41.7±3.23% (control – 88.2±4.46%, $p<0.01$).

During the experiment, a significant recalibration in the relative content of epitheliocytes of different stages of differentiation was observed due to a decrease in the number of cells of the stages V and VI by 14.5% and 18.9%, respec-

Table I. Results of cytological examination of impression smears of MMT at different times from the beginning of modeling of experimental streptozotocin-induced diabetes mellitus (M±m, n=20)

Groups of Animals	Stage of Cell Differentiation (%)						ICD NCR
	I	II	III	IV	V	VI	
Control Group	0	0	0	4.2± 0.24	78.7± 1.15	16.2± 1.18	512.8±9.48
							0.16±0.004
ESID	6.0± 1.14 *	9.5± 1.18 *	27.5± 1.16**	20.2± 1.61**	30.2± 1.33**	6.6± 1.41**	378.9±8.33*
							0.29±0.004*

Note: probable difference with control *p<0.05, **p<0.01;

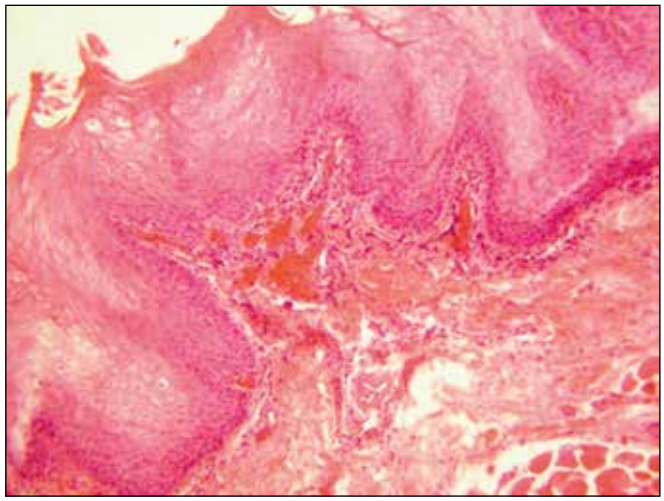

Fig. 2. Uneven thickening of the stratum corneum and hyperkeratosis of the mucous membrane of the tongue 8 weeks after the beginning of ESID modeling. Hematoxylin and eosin staining. Photomicrograph. Magn: x200.

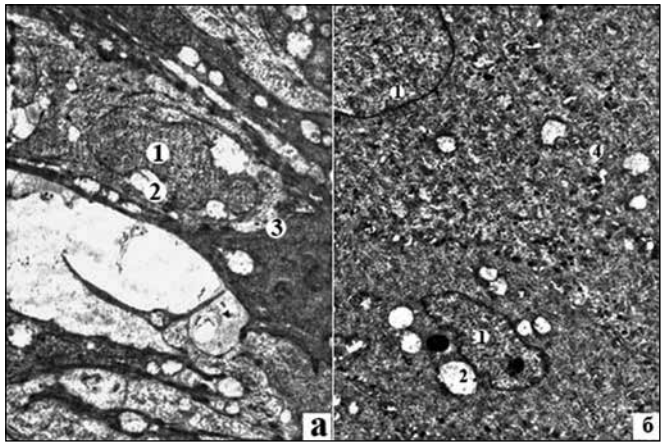

Fig. 3. Vacuolization of the cytoplasm of superficial epitheliocytes and sharp expansion of the intercellular space (a); appearance of a large number of keratohyalin granules, karyopyknosis in granular epitheliocytes (b) 8 weeks after the beginning of ESID modeling. Electronic microphotographs. Magn.: a) x 12000, b) x10000. Designations: 1 – nucleus, 2 – vacuoles, 3 – intercellular contacts, 4 – keratohyalin granules.

tively (p<0.05), with an increase of cells of differentiation stage IV and especially stage III.

Study of impression smears of MMT reveals cells of differentiation stages I-II (Table 1), which significantly affects the changes in the ICD. Its values decrease by 26.1%.

Correlation analysis of morphometric parameters show that the direct proportional relationship between various values is disturbed. At the same time, with increasing cell area, the values of NCR significantly increase (r=0.79, p<0.05).

8 weeks after the beginning of ESID modeling, the number of epitheliocytes of differentiation stages I-III continues to increase (Table 1), and the number of epitheliocytes of differentiation stages IV-VI decreases, which leads to a significant decrease in ICD and increase in NCR. Such changes in impression smears of MMT indicate active processes of epithelial desquamation with increasing duration of ESID.

The analysis of KI values revealed a number of patterns in changes of MMT in ESID. KI decreases to 58.9±3.56, compared with the control (p<0.05), which indicates a disturbance of the processes of epithelial cell differentiation and exposure of deeper layers of MMT.

8 weeks after the beginning of ESID modeling on MMT preparations, the stratum corneum is irregular, sharply thickened, hyperkeratosis is detected, which is characterized by the presence of layers of keratin masses (Fig. 2).

The surface layer of flattened epitheliocytes is thinned and there are cavities in it. The adhesion of microorganisms is revealed in a large number of observations and, mainly, it is diffuse. On superficial epitheliocytes there are mainly coccal forms of microorganisms.

According to tinctorial properties, dark and light epitheliocytes are differentiated among spinous epitheliocytes. In the nuclei of light cells, the nucleoli are not revealed. In dark cells, pyknotically deformed nuclei of irregular shape are often observed. Partially spinous epeliocytes are replaced by fine-grained, heterogeneous material. In the layer of these cells there is an intense transepithelial migration of lymphocytes.

At the ultrastructural level, superficial epitheliocytes form numerous irregular microforms. The vacuolation of the cytoplasm of superficial epitheliocytes and uneven intracellular distribution of keratohyalin are observed. Large granules are found more often, at the same time cytoplasmic accumulations of dusty keratinosomes sometimes occur. In the surface layer during the expansion of the intercellular space between individual epitheliocytes, large vacuoles are formed (Fig. 3 a). Some cells contain nuclei of chimeric shape, many nuclei are in a state of pyknosis.

In the cytoplasm of granular epitheliocytes, thin and short tonofilaments are diffusely distributed, creating the effect of medium osmophilicity. Small single granules of keratohyalin, lipid drops, vesicles of different sizes with small heterogeneous rounded inclusions are detected (Fig. 3b). Some vacuoles look optically transparent. Under electron microscopy, the shape of spinous epitheliocytes is irregular, polygonal, and sometimes flattened. 1-2 to 4-5 vacuoles are often found in their cytoplasm. They contain a fine-grained or electron-light matrix surrounded by a membrane. Spinous epitheliocytes with apoptotic bodies are dominant in some samples. Sometimes, there are single epitheliocytes with signs of complete destruction of most mitochondria.

There is a large number of pinocytic vesicles, bundles of tonofibrils and single granules of glycogen in the cytoplasm of basal cells. Disturbance of intercellular contacts with formation of optically transparent intercellular spaces in which fragments of cytoplasmic processes of the neighbouring epitheliocytes are observed is defined. The basement membrane is thickened, in some areas its integrity is violated and osmophilicity is reduced.

There is a swelling of the lamina propria of MMT. Along with this, intense lymphocyte-leukocyte infiltration is detected, the number and thickness of collagen bundles increase, and they acquire a chaotic orientation. The amount of connective tissue elements is particularly high in the paravasal space, which adversely affects the transport of nutrients to epitheliocytes.

DISCUSSION

As the duration of ESID increases to 8 weeks, the morphological changes of MMT increase and are characterized by a diverse combination of atrophic and hyperplastic processes and as a result, the stratified squamous epithelium thickens unevenly. There are pronounced dystrophic changes in epitheliocytes of the stratum corneum (dyskeratosis, parakeratosis). All areas of MMT are inflamed. The size of spinous cells increases, and their intercellular space decreases. Epithelial growth reaches large size and various shapes.

At all times of observation in the course of ESID, the process of keratinization of the epithelium of MMT undergoes changes. There are structural changes in epithelial cells: vacuolation of the cytoplasm, pyknosis, cytolysis, nuclear exposure, karyorexis, dinuclearity. Some authors [7] revealed accumulation of adipose tissue in the submucosal layer, which, in their opinion, is a specific sign of diabetes.

Cytological studies of impression smears of MMT in ESID showed increased desquamation of epithelial cells, which is confirmed by an increase in the number of epithelial cells of differentiation stages I-III and a decrease in the number of epithelial cells of differentiation stages IV-VI, which leads to a significant decrease in ICD and increased NCR. Such changes lead to an inflammatory reaction of MMT with the subsequent deterioration of the cytological picture due to maladaptation of the cellular response, aimed at decompensating the disorders that develop in ESID [8].

The processes of epithelium differentiation reflected by cytograms, were changed. In particular, there is a decrease in the prevalence of cells of stage IV by an average of 10.2% ($p<0.05$) and, to a lesser extent, of differentiation stage VI against a significant increase in the percentage of epithelial cells of stage V by an average of 18.3% ($p<0.05$). This may indicate a decrease in the regenerative function of the epithelium. In keratinized areas of MMT, the percentage of cells of differentiation stage IV was likely to decrease by an average of 2.4-fold, while the prevalence of non-nuclear scales was likely to be higher than in the control. This indicates an increase in the processes of keratinization.

The KI values had a tendency to increase, which confirms the above data of cytograms, and is also an unfavourable prognostic sign. This was especially pronounced in ESID at the stage of decompensation. Patients with ESID have a tendency to increase keratinization in all areas of MMT [9].

The 8th week of ESID course was characterized by a tendency to reduce the ICD compared with that in control animals, as indicated by other authors [5]. The tinctorial properties of epitheliocytes in ESID were changed. At the same time, the dark and light cells at staining by azure differentiate. The difference in microbial adhesion of these cells is revealed. Dark epitheliocytes are characterized by the presence of an increased number of microorganisms on the surface of plasmolemma. According to our data, tinctorial features explain the differences in the percentage of adhered microorganisms [10, 11].

It was noted that diabetic glossitis has its own morphological specificity, which differs significantly from other inflammatory processes of the oral mucosa. Experimental studies] showed that rats with hyperglycemia have a large amount of epithelial plaque in MMT. This is confirmed by the data of some authors [10, 12] who proved that patients with hyperglycemia have higher levels of pathogenic factors of the oral mucosa, among which are various microorganisms: Candida albicans, Prevotella intermedia, Bacteroides gracilis, Eikenella corodens and the like.

The cytological examination revealed a significant amount of pathological bacterial microflora on the surface of the tongue. Analyzing the literature, it can be concluded that many studies have proven the presence of pathological changes in MMT under the influence of hyperglycemia. In our study, the colonization of MMT was judged by the number of adhered bacterial cells per one epitheliocyte, ie, the adsorption reaction of microorganisms was determined. The colonization of epitheliocytes by microorganisms revealed individual fluctuations in the colonization of the epithelium of the tongue. Decreased resistance to bacteria of MMT in patients with uncontrolled hyperglycemia may be caused by impaired chemotaxis [13] and phagocytosis of neutrophils [14], which are common for diabetes.

CONCLUSIONS

Morphological changes of MMT in ESID are characterized by various combination of atrophic and hyperplastic processes, owing to that the multilayered squamous epithelium is unevenly thickened. There are pronounced dystrophic

changes in the epitheliocytes of the stratum corneum (dyskeratosis, parakeratosis) in the area of the taste buds. All areas of MMT are inflamed which indicates the development of diabetic glossitis.

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The dissertation research was performed within the research work of the Department of Human Anatomy, Operative Surgery and Topographic Anatomy "Morphofunctional Characteristics of Some Organs and Functional Systems in Type 1 Diabetes Mellitus in the Postnatal Period of Ontogenesis" (registration number 0109U001106), which received funding from the Ministry of Health of Ukraine.

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Conflict of interest:
The Authors declare no conflict of interest.

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Received: 11.05.2021
Accepted: 28.08.2021

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article