Changes in the Cytomorphometric Indices in Epitheliocytes of the Oral Mucosa of Patients with Generalized Periodontitis

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Abstract
For the study of some mechanisms of the pathogenesis of generalized periodontitis, there were examined 64 patients who were divided into the following groups: Group I – 12 healthy people (6 men and 6 women); Group II – 40 patients with grade I generalized periodontitis (20 men and 20 women) and Group III – 12 patients with grade II-III generalized periodontitis (6 men and 6 women). On the cytological preparations of buccal epitheliocytes of the oral mucosa stained with aceto-orcein, 100 nucleus-containing epitheliocytes were examined with the help of microscope "Axioskop", a company Zeizz, with a 1000× magnification. Cytomorphometric characteristics were studied: the perimeter and the cell surface area, the perimeter and the surface area of the nucleus, and the ratio of the surface area of the nucleus to the cell surface area.

In all the patients with generalized periodontitis, the perimeter and cell area and the perimeter and epithelial cell nuclei area (p<0.05 – p<0.001) as well as the nuclear-cytoplasmic correlation reduced significantly, especially in men, that could be regarded as compensatory changes at the cellular level. According to the parameters of cells of epitheliocytes, and especially their nuclei, in both healthy people and patients with generalized periodontitis, gender differences were identified, especially in Group III. Thus, it could be argued that the measurement of the perimeter and the area of cells of buccal epitheliocytes and their nuclei reflects the functional activity of the hereditary apparatus in the patients with generalized periodontitis.

Keywords
generalized periodontitis; buccal epitheliocytes; cytomorphometry

Problem statement and analysis of the latest research

Generalized periodontitis (GP), as a multifactorial pathology, is associated with the influence of aggressive exo- and endogenous (including hereditary) factors on the organism. In this case, inflammatory and immune responses, which are important in the pathogenesis of GP, are realized under specific conditions of the environment [1]. Today, the significant role of the hereditary factor in the development of GP is proven by numerous studies. In particular, the genealogical research of pedigrees in patients with GP allowed us to distinguish two variants of the disease development: favourable (without genetic predisposition) and unfavourable (in patients with family history of this disease) [2, 3]. The significant role of the genetic component in the development of GP was proved by the twin method, which is classical for human genetics [4]. On the basis of discriminant and factor analysis of the complex of dermatoglyph indicators, the most informative signs of GP were determined [1, 5]. The genetic predisposition to the development of GP was also
confirmed by the peculiarities of the distribution of blood groups of the the ABO system and Rhesus factor [6] and antigens of the human leukocyte antigen (HLA) blood groups [7, 8], as well as by molecular genetic methods [9, 10]. According to the results of the cytological study of the genetic markers of the functional status of the genotype of somatic cells in patients with GP, impairment of the processes of the implementation of hereditary information at the cellular level were found according to the parameters of euchromatin, chromatization index, nucleolar index and gender chromatin [1, 11]. However, epigenetic mechanisms of GP development have not been yet fully determined.

They are the epigenetic signals, the number of which in the cell is diverse, that control and direct the activation or blocking of certain genes through the methylation and demethylation of cytosine bases of deoxyribonucleic acid (DNA), modification of histone code, transcriptional and translational blocking of genes with small ribonucleic acid (RNA), changes in the conformation of chromatin [12, 13, 14]. Changes in the structure of the latter one (euchromatin/heterochromatin) are interrelated with morphometric cell parameters, since free movement of chromatin depends on the perimeter and area of the nucleus, and indirectly – on cells [15]. In addition, the majority of epigenetic processes are interdependent, which ensures and guarantees the reliability of genetic control over the selective functioning of genes [16, 17].

From this perspective, the cytomorphic analysis of cells and nuclei of epitheliocytes of the oral mucosa (OM), which has not been used in dentistry yet, can be considered as an objective criterion for assessment of the state of cellular metabolism and indirectly characterize some epigenetic mechanisms for the implementation of genetic information that is relevant in pathogenesis of GP.

The objective of the research was to study the cytomorphic parameters of cells and nuclei of buccal epitheliocytes of the OM in patients with chronic GP at various stages of development.

1. Materials and Methods

There were examined 64 patients. Among them, there were 12 (6 men and 6 women) healthy patients (Group I, the control group); 40 (20 men and 20 women) patients with grade I (Group II) and 12 (6 men and 6 women) patients with grade II-III GP (Group III).

The cells and nuclei of buccal epitheliocytes of the OM were studied. The material was taken using a sterile spatula (not earlier than 2-3 hours after eating and brushing the teeth) with a fast, sliding movement along the middle line of the cheek. The depth of the buccal smear made it possible to get cells of the middle layer of the epithelium. The smear was carefully applied to a clean object carrier and was fixed with 96% alcohol during 5-10 minutes. The smear was taken in all the women during the same period of the ovarian-menstrual cycle in a range of three days. On the smear, the DNA was detected using the Feulgen reaction in the modification of L. Ye. Kovalchuk et al. [18]. On each preparation, 100 nucleus-containing epitheliocytes stained with aceto-orcein, were examined, followed by an assessment of their morphometric characteristics. Microscopy was carried out with the aid of a microscope ”Axioskop”, company Zeizz, with 1000× magnification.

Cytomorphic characteristics were studied: the perimeter of the cell, the cell surface area, the perimeter of the nucleus, the surface area of the nucleus and the ratio of the surface area of the nucleus to the cell surface area (the nuclear-cytoplasmic ratio) were calculated. For statistical processing of the results, the parametric methods of descriptive statistics (according to Student’s t-criterion) were used.

2. Results and Discussion

According to the study of the cytomorphic characteristics of buccal epitheliocytes of the OM, in all the patients with GP there was a significant decrease in the perimeter and cell surface area as compared to those in healthy patients (Table 1). Thus, in grade I GP (Group II), these indices decreased convincingly by 6.83% and 14.57%, respectively
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(p<0.05), as compared to the data of the patients in Group I. In the patients of Group III, the decrease in the perimeter and cell surface area was even more pronounced – by 8.89% (p=0.005) and 18.71% (p<0.01), respectively. According to these indices, the difference between the data of Group II and Group III was 1.92% and 3.61%, respectively (p₁ > 0.05).

Since nuclear chromatin is a obligatory substrate for the realization of metabolic functions of cells, its structural-functional transformations, which predetermine the modification of DNA and histone proteins, are very important [19, 20]. The condensation or decomposition of chromatin depends on the morphometric indices of the nuclei, therefore the next stage of the study was the analysis of the perimeter and surface area of epithelial cell nuclei. There was determined a decrease in these parameters depending on the degree of GP development. In particular, the perimeter of the nuclei in Group II reduced significantly – by 9.57% (p=0.005), and the surface area reduced by 15.53% (p<0.05) as compared to the control group. In Group III, this index was also significantly lower – by 13.67% (p=0.001) as compared with that in patients of Group II. There was a significant decrease in the cell surface area in men of Group II – by 21.13% (p<0.001) and Group III – by 30.23% (p<0.001) as compared with healthy ones. The difference between the surface area of cells of patients in Group II and Group III was significant - 7.51% (p₁<0.05).

During the study of the size of the nuclei in men, there was a marked decrease in their perimeter in Group II – by 16.12% (p<0.005), and their surface area – by 26.60% (p=0.001) as compared to healthy people and by 3.74% (p₁=0.05) as compared to the data of the patients in Group II. The surface area of epithelial cell nuclei was also significantly lower – by 29.63% (p=0.01) as compared to the values obtained in Group II.

Therefore, it can be predicted that with the decrease in the surface area of the nuclei, the density of chromatin condensation in their space increased [21]. At the same time, the ability to remodel chromatin and the possibility of releasing individual DNA loci from histone proteins and changing its structure from heterochromatin to euchromatin were suppressed. Although, such process is important to ensure and guarantee the reliability of genetic control over the selective functioning of the genes; however, the reduction in the morphometric indices of the nuclei in GP indirectly confirmed impaired synthesis of polypeptides and, accordingly, adaptive potential of the cell. This assumption was confirmed by the reduced nuclear-cytoplasmic ratio. In patients with grade I GP, it was lower by 3.70%, and in grade II-III GP – by 7.69% as compared to the data received in the group of healthy individuals. The difference between Group II and Group III was 3.85%.

The next stage of the work was the study of gender peculiarities of morphometric indices of epitheliocytes of the OM (Table 2). Men showed a significant decrease in these parameters depending on the degree of the GP development: the perimeter of cells in grade I GP – by 9.03% (p<0.001), in grade II-III GP – by 13.12% (p<0.001) as compared to the control group. In men of Group III, the perimeter was smaller by 3.75% (p₁<0.05) as compared with that in patients of Group II. There was also a significant decrease in the cell surface area in men of Group II – by 21.13% (p<0.001) and Group III – by 30.23% (p<0.001) as compared with healthy ones. The difference between the surface area of cells of patients in Group II and Group III was significant - 7.51% (p₁<0.05).

The nuclear-cytoplasmic ratio in men of Group II reduced by 7.41% as compared to healthy individuals. Although, in Group III, the surface area of cells and nuclei decreased significantly as compared to the normal values; however, the index of their ratio was equal to that in healthy individuals. The above-mentioned facts suggest that in men with a parallel decrease in cell and nuclei parameters, there were deeper impairment of gene expression – from transcription, and posttransplantation processes in the nucleus to suppression of translation in the cytoplasm. [13].

In female patients with GP, most of the studied morphometric parameters tended to decrease;
Table 1. Cytomorphometric indices of epitheliocytes of the OM in healthy individuals and patients with grade I, II-III GP (M±m)

<table>
<thead>
<tr>
<th>Indices</th>
<th>Groups</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group I healthy individuals (the control group) n=12</td>
</tr>
<tr>
<td>Perimeter of cell, µm</td>
<td>354.82±7.28</td>
</tr>
<tr>
<td>Cell surface area, µm²</td>
<td>8089.50±359.89</td>
</tr>
<tr>
<td>Perimeter of the nucleus, µm</td>
<td>58.95±1.31</td>
</tr>
<tr>
<td>Nucleus surface area, µm²</td>
<td>229.18±10.03</td>
</tr>
<tr>
<td>Nuclear-cytoplasmic ratio</td>
<td>0.028</td>
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</tbody>
</table>

Notes. Probability of difference: p – to the value of the indices in Group I; p₁ – to the value of the indices of Group II.

However, significantly less than in men. In grade I GP, the perimeter and the surface area of cells were lower by 4.53% and 7.98% as compared to the control group (p>0.05); in grade II-III GP, these parameters almost did not differ from the data of patients of Group I and Group II (p>0.05; p₁>0.05); in men, they significantly decreased depending on the degree of GP development.

The perimeter and the surface area of epithelial cell nuclei of the OM in women with grade I GP had a slight tendency to decrease as well – by 2.98% and 2.08% as compared to the control group (p>0.05). However, in grade II-III GP, these indices have already changed significantly: the perimeter of the nuclei reduced by 9.93% (p<0.001) as compared to that in healthy individuals and by 6.75% (p₁<0.05) as compared to the data of the patients with grade I GP. The surface area of the nuclei in patients of Group III was significantly lower – by 19.08% (p<0.001) as compared to the normal values, and by 16.66% (p₁<0.005), as compared to the data obtained in women of Group II.

In female patients of Group II, the nuclear-cytoplasmic ratio increased by 7.41% as compared to that in Group I. In Group III, this index was 8.0% lower as compared to the normal values and by 16.0% as compared to the data of Group II.

The comparison of the cytomorphometric parameters in men and women revealed clear gender differences. In particular, healthy men had higher morphometric indices of cells and nuclei of epithelial cells than healthy women, and the index of the nuclear-cytoplasmic ratio (by 7.41%) predominated. In male patients with GP, a significant decrease in the perimeter and the surface area of cells (p<0.001) and the perimeter and the surface area of the nuclei (p<0.005; p≤0.01) were determined, and the indicators worsened with the deepening of the pathological process. In women, the perimeter and the surface area of cells had only a tendency
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to decrease as compared to healthy ones and were the same at different stages of GP development, while the perimeter and the surface area of the nuclei reduced significantly in grade II-III GP only (p<0.001). Another gender peculiarity was the increase in the perimeter and the surface area of epitheliocytes of the OM in women of Group III as compared to Group III.

The obtained data on the gender peculiarities of the dynamics of the cytomorphometric parameters of epithelial cells and their nuclei showed a wider range of normal response of the respective genes in women as compared to men in GP. First of all, this may be due to different epigenetic mechanisms, in particular genomic imprinting at the level of translation and post-translational changes [13].

Thus, we determined changes in the cytomorphometric characteristics of the cells and nuclei of buccal epitheliocytes in GP that deteriorate with the increase of the disease severity (especially in men) affecting the functional state of the hereditary apparatus of patients with GP. They can be regarded as compensatory changes at the cellular level. Thus, changes in the size of epithelial cells are important for the detection of cellular mechanisms in the pathogenesis of GP. It is reasonable to assert that the measurement of the parameters of epithelial cells in combination with the indices of their nuclei reflects the functional activity of the hereditary apparatus in GP, which was proven by scientists studying other human cells [22, 23].

3. Conclusions

1. In patients with GP, the cytomorphometric indices of cells and nuclei of epithelial cells of OM changed significantly: their perimeter and surface area, as well as the nuclear-cytoplasmic ratio decreased significantly in grade I GP (p<0.05; p=0.005) and even more significantly – in grade II-III GP (p=0.005; p<0.01; p=0.001),

2. According to the indices of the perimeter and the surface area of cells of buccal epitheliocytes and, especially, according to the same parameters of their nuclei, in both healthy individuals and patients with GP, there were observed pronounced gender differences. Sexual dimorphism was more manifested in grade II-III GP.

4. Prospects of Further Researches

Prospects for further research in this area are in the study of the densitometric indices in epitheliocytes of the OM in patients with GP.

References

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