

Original article

Reprint

The nature of changes in cell renewal and apoptosis of gingival epithelium in postmenopausal patients with chronic generalized periodontitis

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Abstract: Objective: to identify the nature of proliferation and apoptosis process disorders in the gingival epithelium in postmenopausal women with varying degrees of bone mineralization for early diagnosis and optimization of combination therapy.

Materials and Methods. We examined 80 women aged 55–60 years with chronic generalized periodontitis (CGP) and reduced bone mineral density (BMD). All patients underwent a standard dental examination, including an index assessment of periodontal tissues. BMD was quantified via a densitometric analysis of the bone tissue condition. The investigation of periodontal epithelial cell renewal was performed using immunohistochemical studies.

Results. We discovered that in postmenopausal patients with CGP and BMD disorders, there was a reduction in the proliferation of gingival epithelial cells with activation of their apoptosis (Iapt=0.73±0.03%, Iki-67=11.77±0.27%) vs. the patients with intact periodontium (Iapt 0.29±0.04%, Iki-67=9.88±0.09%), p=0.04.

Conclusion. We discovered that CGP in postmenopausal women occurs with enlarged activity of apoptosis of gingival epithelial cells against the background of their reduced proliferation. The activity of inflammatory destructive processes in periodontal tissues is higher in patients with osteopenia vs. those without BMD impairment.

Keywords: chronic generalized periodontitis, proliferation and apoptosis of gingival epithelial cells, postmenopausal osteopenia, Iki-67, Iapt.

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Introduction

Chronic generalized periodontitis (CGP) is a multifactorial systemic inflammatory disease. It results from a mismatch between the immune response of the body and specific groups of agents causing the periodontitis. This ultimately leads to the resorption of both connective and bone tissue in the periodontal complex accompanied by tooth loss [1–3].

Most cross-sectional studies have confirmed by now the relationship between osteoporosis and periodontitis and convincingly demonstrated their predominantly concomitant course in women [4–8]. One of the proposed explanations is associated with a decline of postmenopausal estrogen content and the lack of its regulatory effect on the immune system [5, 7, 8].

Estrogens play a critical role in periodontal soft tissue functioning and alveolar bone remodeling. This effect is achieved via indirect mechanisms, such as the formation of biogenic substances (growth factors, cytokines). Estrogens activate osteoblast differentiation and osteoclast apoptosis. A reduction in the level of estrogens in the body leads to an increase of the bone tissue resorption under the impact of parathyroid hormone [6, 7].

The conducted studies proved that CGP is accompanied by disorders of the cell renewal processes in the gingival epithelium with simultaneous activation of apoptosis, which negatively affects regeneration processes in periodontal complex tissues [9, 10].

Accrued scientific evidence suggests that some apoptosis factors are highly sensitive and also are of a high predictive value in relation to periodontal disease [11]. The Ki-67 protein is a regulatory protein found in a mitotic cell. The determination of the Ki-67 protein content is used to assess the intensity of cell renewal, while its correlation with apoptosis makes it possible to elucidate the mechanisms of regeneration in inflammatory periodontal diseases [9].

Objective – to identify the nature of disorders in proliferation and apoptosis processes of the gingival epithelium in postmenopausal women with varying degrees of bone mineralization for early diagnosis and optimization of combination therapy.

Materials and Methods

The research was carried out in compliance with the standards of Good Clinical Practice and the Declaration of

Helsinki principles. The study protocol was approved by the Ethics Committee at Penza Institute for Postgraduate Medical Education, a branch of the Russian Medical Academy of Continuing Professional Education of the Russian Federation Ministry of Healthcare. Written informed consent was obtained from all participants prior to their inclusion in the study. A total of 80 postmenopausal women 55–60 years of age were examined. They were distributed among four groups with 20 subjects in each: Group I including patients with CGP against the background of osteopenia with bone mineral density (BMD) readings of -1 to -1.5 SD (standard deviation); Group II comprising subjects with CGP against the background of osteopenia with BMD of -1.5 to -2.5 SD; Group III (the comparison group) encompassing study subjects with CGP and normal BMD values; and Group IV (the control group) consisting of 20 women with intact periodontium and normal BMD values.

The study did not involve women with dentoalveolar anomalies, deformities and complete adentia; oncological diseases; decompensated diseases of the liver, kidneys and cardiovascular system; autoimmune diseases; diabetes mellitus; obesity; and taking glucocorticoids as part of the combination therapy of somatic pathology, or hormone replacement therapy medicines.

All patients underwent a standard dental examination, including an index assessment of periodontal tissues: OHI-S (Oral Hygiene Index-Simplified; J.C. Green, J.R. Vermillion [1964]); Mühlemann Papillary Bleeding Index (PBI) modified by R. Cowell (1975); Gingival Index PMA (P, interdental papilla; M, marginal gingiva; A, attached gingiva) (Parma [1964]); and depth measurements of periodontal pockets [12]. Periodontal diseases were diagnosed in accordance with clinical guidelines. The postmenopause was verified by a gynecologist.

BMD was quantified via a densitometric analysis of the bone tissue condition using an X-ray absorption densitometry. The severity of osteopenia was assessed by T-score.

The study of cell renewal indicators in periodontal epithelial cells was carried out using immunohistochemical examination. The material was represented by biopsy specimens sampled during the extraction of teeth from the marginal part of the gums. Monoclonal mouse antibodies to the Ki-67 protein (Sigma, St. Louis, USA, titer of 1:200) were used. Proliferation index, Iki-67, was calculated by the formula: $Iki-67(\%) = \text{number of positively stained cell nuclei} / \text{total number of counted cells} \times 100$.

Cell death in the form of apoptosis was determined using the apoptotic index, Iapt, calculated according to the formula: $Iapt(\%) = Na / N \times 100$ (number of apoptotic nuclei stained via the standard method of hematoxylin and eosin / total number of nuclei) $\times 100$. The number of expressing cells was counted in 30 fields of view at the standard magnification, and the digital data were recalculated per 1 mm² using the Videotest-Morphology 4.0 applied morphometric software package.

The obtained results were processed using the SPSS version 21 and Microsoft Office Excel 2010 application package. To examine the distributions of all examined parameters, we employed the Kolmogorov-Smirnov and Shapiro-Wilk tests. The Kruskal-Wallis test was used to examine intergroup differences. Our main results were presented in the form of $M \pm m$, where M is the mean, and m is the error. The results were assumed statistically significant

if the probability of an error did not exceed 5% ($p \leq 0.05$). Spearman non-parametric tests were applied to establish the correlation between quantities.

Results

In patients of the control group with intact periodontium, the following values of proliferative and antiapoptotic activity of gingival epithelial cells were identified: Iki-67= $9.88 \pm 0.09\%$, Iapt= $0.29 \pm 0.04\%$ at normal BMD ($T = -0.22 \pm 0.06$ SD).

In the comparison group, in postmenopausal patients with CGP against the background of normal BMD ($T = -0.55 \pm 0.09$ SD), we observed an increase in the proliferation index (Iki-67= $21.27 \pm 0.83\%$) against the background of a low apoptosis rate (Iapt= $0.60 \pm 0.10\%$). Performed correlation analysis established a direct correlation between the values of PMA and Iapt indices ($r = 0.89$), along with an inverse correlation with Iki-67 ($r = -0.79$).

In Group I (subjects with CGP) against the background of osteopenia ($T = -1.23 \pm 0.05$ SD), we observed a progressive lag of epithelial cell proliferation processes from their apoptotic activity, which was reflected in an increase of the apoptotic index value (Iapt = $0.73 \pm 0.03\%$) and a decrease in the proliferation index value (Iki-67= $11.77 \pm 0.27\%$). These indicators were statistically significantly different from those in the control group (Iki-67= $9.88 \pm 0.09\%$, Iapt= $0.29 \pm 0.04\%$, $p = 0.04$) but had no significant differences from the comparison group ($p = 0.07$).

In Group II, CGP also occurred against the background of osteopenia ($T = -1.7 \pm 0.06$ SD). Further inhibition of the proliferative activity of epithelial cells (Iki-67= $7.21 \pm 0.20\%$) was noted with a twofold increase in apoptosis (Iapt= $1.53 \pm 0.38\%$), compared with Group I. At the same time, there was a direct correlation between the values of BMD and Iapt ($r = 0.65$), and an inverse correlation with the values of Iki-67 ($r = -0.73$).

Discussion

Our study results demonstrated that in postmenopausal patients with CGP and without BMD disorders, there was a reduced proliferation of gingival epithelial cells with the activation of their apoptosis as compared to patients with intact periodontium. This finding is explained by the fact that DNA is damaged in a significant part of cells during inflammation, and, as a result, they undergo apoptosis. We established a direct correlation between the values of PMA index and apoptotic (Iapt) indices, and an inverse correlation of the former with the proliferation index (Iki-67), which implied the relationship and mutually aggravating effect between inflammatory destructive processes in the periodontal complex tissues and the degree of cell renewal process disorder in the epithelium of the gingival mucosa.

In postmenopausal patients with CGP and severe BMD disorders, more significant disorders in the proliferation and apoptosis of gingival epithelial cells were identified with significant differences in both indicator values from the values obtained for the patients with CGP and without BMD impairment, and for those with less pronounced indicators of osteopenia in terms of proliferation and apoptosis.

The obtained results, along with available published data, allow concluding that the proliferative activity and apoptosis of gingival epithelial cells are controlled by local and systemic

factors. Some apoptotic factors are highly sensitive and have high positive predictive value for periodontal disease [11].

Conclusion

Our major findings are as follows. In postmenopausal women, CGP occurs with enlarged activity of gingival epithelial cell apoptosis against the background of their reduced proliferation. The activity of inflammatory destructive processes in periodontal tissues is higher in patients with osteopenia, compared with patients without BMD impairment. The obtained results should be taken into account for early diagnosis and optimization of combination therapy in postmenopausal women with CGP and impaired BMD.

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