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IMMUNOMORPHOLOGICAL ASPECTS OF CHANGES OF PERIODONTIUM IN PATIENTS WITH CARDIOPULMONARY PATHOLOGY

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The protective properties of the gum depend on the general condition of the body, the presence of diseases of internal organs and systems. Numerous data have been accumulated on the relationship of diseases of internal organs with dystrophic-inflammatory lesions of periodontium. It is known that impaired control of cell death and proliferation leads to disruption of cellular homeostasis and the progression of chronic diseases.

***Objective** - to determine the parameters of the ratio of the processes of proliferation and apoptosis in patients with COPD in combination with CHD.*

***Materials and methods.** A cytological, morphological and immunohistochemical study of gingival epitheliocytes is conducted in 130 patients with COPD in combination with CHD, 38 patients with COPD, and 71 patients with CHD. Control group included 20 somatically healthy patients. Cytological examination was carried out on scrapings from the surface of the epithelium of the attached gingiva, followed by staining and study under light microscope Micros (Austria). Indirect immunocytochemical peroxidase method was used to study a relative amount of immunocompetent cells with CD95 + markers. To study the proliferation marker Ki-67 we used indirect immunocytochemical method with monoclonal antibodies of a biopsy gum material. Statistical processing was carried out using the software "SPSS 13" with the calculation of the median and interquartile range.*

***Results.** Main group showed increase in the percentage of cells at the final stages of differentiation compared with other groups, increased number of cells carrying the receptor for programmed cell death. However, in patients with ischemic heart disease, the proliferative activity of gingival epitheliocytes is increased by 3.7 times compared with the value in a group of practically healthy individuals and is characterized by delayed apoptosis.*

***Conclusion.** Cellular renewal of epithelial cells in this category of patients is characterized by a progressive delay of apoptosis of the epitheliocytes of the oral mucosa from the rate of proliferative processes.*

***Key words:** periodontium, programmed cell death, epithelial cell, proliferation of epithelial cells.*

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ІМУНОМОРФОЛОГІЧНІ АСПЕКТИ ЗМІН У ПАРОДОНТІ ХВОРИХ ІЗ КАРДІОПУЛЬМОНАЛЬНОЮ ПАТОЛОГІЄЮ

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Захисні властивості ясен залежать від загального стану організму, наявності захворювань внутрішніх органів та систем. Накопичені численні дані про взаємозв'язок хвороб внутрішніх органів з дістрофічно- запальними ураженнями пародонту. Відомо, що порушення контролю клітинної загибелі та проліферації призводить до порушення клітинного гомеостазу і прогресуванню хронічних захворювань.

***Мета роботи** - визначити параметри співвідношення процесів проліферації та апоптозу у пацієнтів з ХОЗЛ в поєднанні з ІХС.*

***Матеріал і методи**. Проведено цитологічне, морфологічне та імуногістохімічне дослідження епітеліоцитів ясен 130 пацієнтів з ХОЗЛ у поєднанні з ІХС, 38 хворих з ХОЗЛ, та 71 пацієнта з ІХС. Група контролю - 20 соматично здорових пацієнта. Цитологічне дослідження проводили на зішкрябах з поверхні епітелію прикріплених ясен з подальшим фарбуванням і прогляданням на світловому мікроскопі Micros (Австрія). За допомогою непрямого імуноцитохімічного пероксидазного методу визначали відносну кількість імунокомпетентних клітин з маркерами CD95 +. Для дослідження маркера проліферації Ki-67 застосовували непрямий иммуноцитохімічний метод з використанням моноклональних антитіл з біопсії ясен. Статистична обробка проводилася за допомогою програмного забезпечення "SPSS 13" з розрахунком медіани і інтерквартільного розмаху.*

***Результати**. В основній групі спостерігалося збільшення відсотка клітин, що знаходяться на заключних стадіях диференціювання при порівнянні з іншими групами, збільшення числа клітин, які мають рецептор запрограмованої клітинної*

***Ключевые слова:** пародонт, запрограммированная клеточная смерть, эпителиоцит, пролиферация эпителиоцитов*

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смерті. Однак, у пацієнтів з ІХС проліферативна активність епітеліоцитів ясен підвищується в 3,7 разів у порівнянні зі значенням в групі практично здорових осіб і характеризується відставанням апоптоза.

Висновки. *Клітинне оновлення епітеліоцитів у даної категорії хворих характеризується прогресуючим відставанням апоптозу епітеліоцитів слизової оболонки порожнини рота від швидкості проліферативних процесів.*

Ключевые слова: *пародонт, запрограммированная клеточная смерть, эпителиоцит, пролиферация эпителиоцитов*

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ИММУНОМОРФОЛОГИЧЕСКИЕ АСПЕКТЫ ИЗМЕНЕНИЙ В ПАРОДОНТЕ У ПАЦИЕНТОВ С КАРДИОПУЛЬМОНАЛЬНОЙ ПАТОЛОГИЕЙ

Н.Ю. Емельянова

Защитные свойства десны зависят от общего состояния организма, наличия заболеваний внутренних органов и систем. Накоплены многочисленные данные о взаимосвязи болезней внутренних органов с дистрофически- воспалительными поражениями пародонта. Известно, что нарушение контроля клеточной гибели и пролиферации приводит к нарушению клеточного гомеостаза и прогрессированию хронических заболеваний.

Цель работы *- определить параметры соотношения процессов пролиферации и апоптоза у пациентов с ХОБЛ в сочетании с ИБС.*

Материалы и методы. *Проведено цитологическое, морфологическое и иммуногистохимическое исследование эпителиоцитов десны 130 пациентов с ХОБЛ в сочетании с ИБС, 38 больных с ХОБЛ, и 71 пациента с ИБС. Группа контроля 20 соматически здоровых пациента. Цитологическое исследование проводили на соскобах с поверхности эпителия прикрепленной десны с последующим окрашиванием и просматриванием на световом микроскопе Micros (Австрия). С помощью непрямого иммуноцитохимического пероксидазного метода определяли относительное количество иммунокомпетентных клеток с маркерами CD95+. Для исследования маркера пролиферации Ki-67 применяли непрямой иммуноцитохимический метод с использованием моноклональных антител биопсийного материала десны. Статистическая обработка проводилась при помощи программного обеспечения "SPSS 13" с расчётом медианы и интерквартильного размаха.*

Результаты*. В основной группе наблюдалось увеличение процента клеток, находящихся на заключительных стадиях дифференцировки при сравнении с другими группами, увеличение числа клеток, несущих рецептор запрограммированной клеточной смерти. Однако, у пациентов с ИБС пролиферативная активность эпителиоцитов десны повышается в 3,7 раз по сравнению со значением в группе практически здоровых лиц и характеризуется отставанием апоптоза.*

Выводы. *Клеточное обновление эпителиоцитов у данной категории больных характеризуется прогрессирующим отставанием апоптоза эпителиоцитов слизистой оболочки полости рта от скорости пролиферативных процессов.*

Introduction

The increasing frequency of periodontal disease in systemic changes in the body, the tendency to prolonged and slightly damped flow determine the need for improving the methods of diagnosis and treatment in dentistry [1-2]. Bronchopulmonary and cardiac pathology (chronic obstructive pulmonary diseases (COPD) and ischemic heart disease (CHD) significantly increase the course of diseases of the oral mucosa, in particular periodontium, and they in turn disrupt the functions of the body systems, and they have a mutually affecting effect [3-5].

The epithelium of the gum performs protective barrier function for a complex of periodontal tissues and is provided by its proliferative activity and the ratio of the processes of proliferation and differentiation. It is known that with certain functional states in the body, the intensity of sloughing can exceed the rate of renewal of the deep layers of the gingival epithelium, and activation of apoptosis occurs against sharp inhibition of the proliferative activity of the epithelium. An important role in the evaluation of cellular homeostasis disorders in the gingival epitheliocytes in periodontal diseases belongs to the Ki-67 marker, mast cells and CD 95 (apoptosis marker) [6].

However, the data explaining the processes of the ratio of proliferation and apoptosis in the tissues of the gum in cardio-respiratory diseases are not numerous in the literature. Studying them will not only improve early diagnosis, but also optimize the methods of prevention and treatment of changes in periodontitis in this category of patients.

Study **objective** was to determine the parameters of the ratio of the processes of proliferation and apoptosis in patients with COPD in combination with CHD.

Materials and methods

The study was conducted in the State Institution "National Institute of Therapy named after L.T. Malaya of the National Academy of Medical Sciences of Ukraine". A

total of 239 patients were selected: main group consisted of 130 patients with a verified diagnosis of COPD in combination with CHD, comparison group 1 was represented by 38 patients diagnosed with COPD, and comparison group 2 included 71 patients with a verified diagnosis of CHD.

The control group was represented by 20 patients, representative by sex and age, who did not have a somatic pathology and at the time of the study did not take any medications.

The diagnosis of COPD was established according to the order of the Ministry of Health of Ukraine No. 555 as of 27 Jun 2013 and the provisions set forth in the GOLD (Global Initiative for Chronic Obstructive Lung Disease) 2011-2015 [7]. According to the recommendations of the European Society of Cardiology, the diagnosis of CHD was verified on the basis of a clinic, tests with exercise, Holter monitoring, ECG and coronary angiography.

The main criteria inclusions criteria:

1. Age from 35 to 65 years;

2. Absence of severe concomitant pathology (malignant neoplasms, type II diabetes, chronic pyelonephritis, chronic glomerulonephritis, thyroid disease, anemia);

3.Absence of previous courses of treatment of periodontal tissue within the last 6 months before the beginning of the examination.

Patients underwent complex clinical dental examination. The color and structure of the gingival mucosa were assessed with the degree of gums bleeding according to history and by Muehlemann; depth of periodontal pockets and pathological mobility of teeth. Also index assessment of the condition of periodontal tissues was carried out (simplified Green- Vermilion hygiene index, papillary-marginal-alveolar index (PMA) and periodontal index (PI).

Cytological studies were carried out on scrapings from the surface of the epithelium of the attached gingiva. The cytological preparations were stained with azur-eosin and examined under light microscope Micros (Austria). In order to obtain cell photographs, a CAM 2800 digital video camera was used with light microscopy (lenses x40, eyepiece x10).

Indirect immunocytochemical peroxidase method determined relative amount of immunocompetent cells with CD95 + markers. The resulting preparations were examined under light microscope to determine the antigen-positive cells.

For morphological and immunocytochemical study of marker Ki-67 we used indirect immunohistochemical method with monoclonal antibodies. Study was paraffin blocks from a biopsy gum material taken from the apex of the interdental papilla after anesthesia in 94 patients (49 from the main group, 16 patients with COPD, 22 patients with CHD and 7 patients from the control group).

The statistical processing was carried out using the software "SPSS 13". Since, according to Kolmogorov-Smirnov's test, the law of data distribution did not correspond to the normal one, the median and interquartile range were calculated, Mann-Whitney test was used to compare the quantitative values, and the -square test was used to compare the qualitative values.

Results and discussion

Study of the morphological pattern of scrapings of the gingival epithelium revealed that in the group of somatically healthy patients the main layer consisted of cells of the intermediate layer, whereas cells belonging to the parabasal layer were completely absent (Table 1).

The greatest pathological changes were typical for patients diagnosed with COPD in combination with CHD. In this group, the frequency of occurrence of cells at the final stages of differentiation (superficial and keratinizing) was significantly higher compared with both the control group and the comparison groups. The increase in the number of mature cells was associated with a decrease of the number of the main formation (intermediate cells).

Carrying out typing of gingival leukocytes on the

Table 1

Differentiation of epitheliocytes in patients of study groups

Value	Control	Main group	Comparison group 1	Comparison group 2
Parabasal	0.00 (0.00; 0.00)	0.00 (0.00; 1.00) p=0.007	0.00 (0.00; 1.00) p=0.013	0.00 (0.00; 0.00)
Intermediate	59.00 (56.25; 61.00)	17.00 (15.00; 20.00) p=0.001 p1=0.001 p2=0.001	24.00 (20.50; 29.50) p=0.001	45.00 (42.00; 47.00) p=0.001
Superficial	11.00 (10.00; 13.75)	36.00 (33.00; 39.00) p=0.001 p1=0.003 p2=0.001	33.00 (29.00; 37.25) p=0.001	21.00 (18.00; 22.00) p=0.001
Squamous	30.00 (28.00; 31.75)	46.00 (44.00; 48.00) p=0.001 p1=0.001 p2=0.001	41.00 (38.00; 47.25) p=0.001	34.00 (32.00; 38.00) p=0.001

Note: p - the level of significance of a reliable difference compared with the control, p 1 and p 2 - significance level of significant difference compared with group 1 and 2

expression of differentiation clusters on their surfaces (CD markers) showed that in all groups the number of cells carrying the programmed cell death receptor (CD95 +) is increased, and the most significant increase in the expression of this indicator was observed in the group of patients with COPD and COPD in combination with CHD, which exceeded the control values by more than 2 times (p = 0.001).

An increase of lymphocytes expressing the receptors of induction of apoptosis in the peripheral blood content of patients with COPD leads to its amplification, and may be the cause of the development of T-cell deficiency.

As a result of the study, it was found that in the group of somatically healthy patients, the gingival epitheliocytes showed a low potential of proliferative and antiapoptotic activity for Ki-67 = 1.575 (1.176, 1.883); CD95 + = 19.50 (18.00, 23.75) (Table 2).

Evaluation of Ki-67 marker expression showed an increase in the number of proliferating cells in all groups of patients with somatic pathology. In patients with a verified diagnosis of CHD, the proliferative activity of the gingival epitheliocytes increases by 3.7 times in comparison with the group of practically healthy individuals, however, their apoptosis index is less significant (1.6 times).

However, in patients with COPD and COPD with CHD,

Table 2

Proliferation index in patients of all groups

Value	Main group (n=49)	Comparison group 1 (n=16)	Comparison group 2 (n=22)	Control (n=7)
Ki 67 (μm2)	37818,896 (31767.050;48913.028) p=0.004	41727,164 (14217.487;49094.46) p=0.066	45340,325 (31541.987;65923.187) p1=0.001	14476.419 (10553.686;18483.292)
Ki-67 Area	4,264 (1.339;5.861) p=0.029	5.206 (4.617;6.082) p=0.001	5.941 (5.146;7.082) p=0.001 p1=0.001	1.575 (1.176;1.883)

Note: p - the level of significance of a reliable difference compared with the control, p 1 - significance level of significant difference compared with main group

there is an increase not only in the proliferative capacity, but also percentage of death of epithelial gum cells in the form of apoptosis compared with the values of practically healthy patients. Nevertheless, results for all groups showed significant delay in the processes of apoptosis from the rate of proliferation. Consequently, in the epithelium of the gums in patients with such pathologies as COPD and CHD, proliferative processes are predominate.

Conclusions

According to the obtained data for COPD and COPD in combination with CHD, mechanisms that stimulate the terminal stages of cell differentiation (maturation) are activated to a greater extent than for CHD alone with the decrease in the main formation, which leads to the development of destructive changes in the periodontal complex. Cellular renewal of gingival epitheliocytes in patients with respiratory-cardiac pathology is characterized by a progressive delay of the apoptosis of the gingival epitheliocytes from the rate of proliferative processes.

Perspective of further research

In this regard, the evaluation of the pathological impact of somatic pathology on gingival tissue appears to be relevant which would allow developing adequate methods for early diagnosis and prevention of emerging pathological changes.

There is no conflict of interest.

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