

Abstract

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**CYTOMORPHOMETRIC CHANGES IN GINGIVAL
EPITHELIOCYTES IN PATIENTS WITH CHRONIC
OBSTRUCTIVE PULMONARY IN COMBINATION
WITH CORONARY HEART DISEASE**

Introduction and purpose. This article is devoted to the actual problem of dentistry, namely, cytomorphometric changes in the gingival epithelium of patients with cardio-respiratory pathology. The purpose is to study the cytological and morphometric parameters of gingival epitheliocytes in patients with chronic obstructive pulmonary disease in combination with coronary heart disease.

Materials and methods. 239 patients were examined: the main group, 130 patients with COPD in combination with chronic obstructive pulmonary disease with coronary heart disease; comparison group 1: 38 patients diagnosed with chronic obstructive pulmonary disease; and the comparison group 2: 71 patients with coronary heart disease. The control group is represented by 20 somatically healthy patients.

Morphometric and cytological studies were performed on scrapings of gingival epithelium according to standard procedure with staining with azur-eosin and studying under light microscope.

Results and discussion: It is noted that in patients with co-morbid pathology, the cells at in the late stages of differentiation are more common. Clear leukocyte infiltration is noted in the preparations, and morphometric analysis shows significant decrease in the nuclear-cytoplasmic ratio in patients of all groups due to an increase in the epithelial cell nucleus ($p < 0.05$), with no significant changes in the cell cytoplasm.

Conclusions: This fact justifies the need for early diagnosis of possible violations in the oral cavity, as well as the development of an algorithm for their prevention and treatment.

Keywords: periodontal, gingival epitheliocytes, coronary heart disease, chronic obstructive pulmonary disease, morphometry.

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Резюме

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**ЦИТОМОРФОМЕТРИЧНІ ЗМІНИ ЕПІТЕЛІОЦИТІВ ЯСЕН
У ПАЦІЄНТІВ З ХРОНІЧНИМИ ОБСТРУКТИВНИМИ
ЗАХВОРЮВАННЯМИ ЛЕГЕНІВ У ПОЄДНАНІ
З ІШЕМІЧНОЮ ХВОРОБОЮ СЕРЦЯ**

Мета: вивчення цитологічних і морфометричних параметрів епітеліоцитів ясен у пацієнтів з респіраторно-кардіальною патологією.

Матеріали і методи: обстежено 239 хворих: основна група 130 пацієнтів з хронічними обструктивними захворюваннями легень у поєднанні з ішемічною хворобою серця, група порівняння №1 – 38 хворих з хронічними обструктивними захворюваннями легень, та група порівняння №2 – 71 пацієнт з ішемічною хворобою серця. Група контролю – 20 соматично здорових пацієнтів.

Морфометричні та цитологічні вивчення проводили на зішкрябах епітелію ясен за стандартною методикою з фарбуванням препаратів та вивченням їх на світловому мікроскопі.

Результати та їх обговорення: у пацієнтів з поєднаною патологією частіше зустрічалися клітини, які перебувають на пізніх стадіях диференціювання. Також в препаратах відзначалася явна лейкоцитарна інфільтрація, а морфометричний аналіз показав вірогідне зниження ядерно/цитоплазматичного співвідношення у пацієнтів всіх груп за рахунок збільшення площі ядра епітеліоцитів ($p < 0,05$) на тлі відсутності істотних змін площі цитоплазми клітини.

Висновки: даний факт обґрунтовує необхідність ранньої діагностики можливих порушень в порожнині рота, а також розробки алгоритму їх профілактики та лікування.

Ключові слова: пародонт, епітеліоцити ясен, ішемічна хвороба серця, хронічна обструктивна хвороба легень, морфометрія.

Резюме

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ЦИТОМОРФОМЕТРИЧЕСКИЕ ИЗМЕНЕНИЯ ЭПИТЕЛИОЦИТОВ ДЕСНЫ У ПАЦИЕНТОВ С ХРОНИЧЕСКИМИ ОБСТРУКТИВНЫМИ БОЛЕЗНЯМИ ЛЕГКИХ В СОЧЕТАНИИ С ИШЕМИЧЕСКОЙ БОЛЕЗНЬЮ СЕРДЦА

Цель: изучение цитологических и морфометрических параметров эпителиоцитов десны у пациентов респираторно-кардиальной патологией.

Материалы и методы: обследовано 239 больных: основная группа 130 пациентов с хроническими обструктивными заболеваниями легких в сочетании с ишемической болезнью сердца, группа сравнения №1 – 38 больных с хроническими обструктивными заболеваниями легких, и группа сравнения №2 – 71 пациент с ишемической болезнью сердца. Группа контроля – 20 соматически здоровых пациентов.

Морфометрические и цитологические изучения проводили на соскобах эпителия десен по стандартной методике с окрашиванием препаратов и изучением на световом микроскопе.

Результаты и их обсуждение: у пациентов с сочетанной патологией чаще встречались клетки, находящиеся на поздних стадиях дифференцировки. Также в препаратах отмечалась явная лейкоцитарная инфильтрация, а морфометрический анализ показал достоверное снижение ядерно/цитоплазматического соотношения у пациентов всех групп за счет увеличения площади ядра эпителиоцитов ($p < 0,05$) на фоне отсутствия существенных изменений площади цитоплазмы клетки.

Выводы: данный факт обосновывает необходимость ранней диагностики возможных нарушений в полости рта, а также разработки алгоритма их профилактики и лечения.

Ключевые слова: пародонт, эпителиоциты десны, ишемическая болезнь сердца, хроническая обструктивная болезнь легких, морфометрия.

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Introduction

The influence of general somatic pathology on the periodontal complex lesion acquires particular relevance which is associated with its influence on the leading links in the pathogenesis of gum tissue diseases [1].

The great interest of dentists is connected with the study of periodontal diseases in patients with coronary heart disease (CHD) and chronic obstructive pulmonary diseases (COPD), as well as their combination, which is associated with the presence of similar links of pathogenesis [2–3].

Existing clinical methods of periodontal examination do not always correspond to the true picture, since early changes in gingival tissue during visual examination may be absent. However, non-invasive study methods with exfoliative cytology increase the possibility of earlier detection of different damages of the periodontal complex [4].

Consequently, the problem of improving the diagnosis of early manifestations of periodontal pathology in patients with pulmonary cardiac pathology is quite relevant not only for dentists, but also for internist doctors.

The aim of our work was to study the cytological and morphometric parameters of gingival epitheliocytes 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: the main group consisted of 130 patients with verified COPD diagnosis in combination with coronary artery disease, comparison group 1 included 38 patients diagnosed with COPD, and the comparison group 2 – 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.

Morphometric and cytological studies were carried out on the scrapings of the gingival epithelium by a standard procedure – scraping of the surface of the epithelium of the attached gingiva was carried out using a dental spatula with transfer to the slide and subsequent drying of the smears. The cytological preparations were stained with azure-eosin and examined under light microscope Micros (Austria). In order to obtain cell photographs, a CAM 2800 digital video camera was used with light microscop-

py (lenses x40, eyepiece x10). BioVision computerized morphometric program was used to determine the morphometry of cells (perimeter, nucleus and cytoplasm area, volume percentage of cellular elements in gum preparations) [5]. The frequency of cells with anomalies was determined per 1000 cells. To study the cellular composition (monocytes, neutrophils, lymphocytes), 50 images of cells with clear boundaries were selected.

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 χ -square test was used to compare the qualitative values.

Results and discussion

It should be immediately noted that the cytomorphometric data of the control group were estimated as the norm, since their indicators completely coincided with those that are indicated in the modern scientific literature. Cells of these patients, in more than 60.0% of cases, had an unpainted cytoplasm with a compact nucleus, and the remaining percentage was represented by superficial, denucleated cells. Preparations included cells of an intermediate type with a sufficiently large nucleus and a weakly basophilic cytoplasm. Epitheliocytes of the parabasal layer were not observed.

However, study of scrapings from the gums of persons in the study groups noted that more than 90.0% of patients had pathological changes that were most pronounced in patients with combined cardio-respiratory pathology (Table 1). In the same group, the cells that are at the last stages of differentiation (keratinizing and superficial) were more often not only compared with somatically healthy patients, but also with the comparison groups 1 and 2. With the increase in the growth of mature cells, the number of cells in the main formation (intermediate) in all groups decreased in comparison with the norm values. It should be noted that in with history of COPD (main group and comparison group 1), we noted epithelial cells belonging to the parabasal layer which is not typical for the epithelium of healthy patients, as well as for patients with CHD.

Carrying out a cytological study, it was established that the number of leukocytes in the preparations of the gingival epithelium of the control patients completely corresponds to the normal parameters, since lymphocytes always appear in the gin-

gival epithelium, but in a small amount. The analysis of the percentage of lymphocytes, monocytes and neutrophils in patients without somatic pathology was normal.

At the same time, in patients of all groups (mainly in the group of patients with COPD) cytological preparations showed clear leukocyte infiltra-

tion which had differences not only from control, but also from the patients of the main group and the group of patients with CHD. And the percentage of lymphocytes, neutrophils and monocytes indicated an approximation to the data observed for periodontal diseases accompanied by inflammation.

Table 1 – Distribution of epithelial cells according to the degree of their differentiation

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; p1 and p2 – significance level of significant difference compared with group 1 and 2

The obtained morphological parameters of the gingival epitheliocytes of somatically healthy patients completely correspond to those given in the modern literature, which allowed us to use them as a criterion for an adequate evaluation of similar parameters in the remaining patients. In the control group, the mean values of the perimeter of the nucleus (Pn) were 39.085 [37.100, 42.213] μm , the perimeter of the cell (Pc) was 220.395 [209.548, 238.980] μm , and the ratio of Pn to Pc reached 0.111 [0.160, 0.188] μm . Nucleus area (An) was 90.205 [78.820, 103.283] μm^2 , cytoplasm area (Act) was 2963,270 [2601.068, 3479.565] μm^2 , and the ratio of An to Act was 0.030 [0.028, 0.032] μm^2 .

However, the determination of morphometric parameters in the patients of the studied groups

made it possible to record a significant decrease in all morphometric cell values, especially the nuclear/cytoplasmic ratio due to the increase in the epithelial cell nucleus against the absence of significant changes in the cell cytoplasm (Table 2).

The increase in the size of the nucleus is associated with a change in the duration (decrease) of the life of the epithelial cell and the restriction of the production of young cells, resulting in the increase of percentage of mature and old cells within the cell stratum.

Correlation analysis identified the relationships between the morphometric parameters. A positive correlation of the nucleus perimeter and area was observed in all the study groups (Table 3).

Table 2 – Morphometric parameters of epitheliocytes of the studied groups

Parameter	Main group	Comparison group 1	Comparison group 2	Control
Pn, μm	45.220 (40.758; 46.676) p = 0.001	43.849 (41.544; 45.578) p = 0.001	44.098 (40.772; 46.148) p = 0.001	39.085 (37.100; 42.213)
Pc, μm	190.334 (170.651; 200.084) p = 0.001; p1 = 0.001 p2 = 0.001	203.032 (196.755; 223.898) p = 0.003	214.380 (196.776; 224.602) p = 0.037	220.395 (209.548; 238.980)
Pn/Pc	0.235 (0.224; 0.252) p = 0.001; p1 = 0.001 p2 = 0.001	0.209 (0.200; 0.223) p = 0.001	0.210 (0.202; 0.224) p = 0.001	0.170 (0.160; 0.180)
An, μm^2	118.114 (85.792; 124.989) p = 0.001	111.005 (105.256; 120.061) p = 0.001	106.760 (93.514; 121.188) p = 0.001	90.205 (78.820; 103.283)
Ac, μm^2	2221.268 (1879.042; 2433.080) p = 0.001; p1 = 0.001 p2 = 0.001	2437.956 (2256.342; 2961.526) p = 0.001	2821.426 (2316.256; 3278.850) p = 0.009	3062.905 (2682.123; 3593.725)
Act, μm^2	2149.235 (1810.248; 2348.725) p = 0.001; p1 = 0.010 p2 = 0.001	2313.402 (2072.767; 2586.824) p = 0.001	2718.006 (2193.080; 3183.190) p = 0.006	2963.270 (2601.068; 3479.565)
An/Act	0.059 (0.048; 0.064) p = 0.001; p1 = 0.001 p2 = 0.001	0.051 (0.014; 0.046) p = 0.001	0.042 (0.038; 0.046) p = 0.001	0.030 (0.028; 0.032)

Note: p – the level of significance of a reliable difference compared with the control; p1 and p2 – significance level of significant difference compared with group 1 and 2

Table 3 –The presence of interrelations between the morphometric parameters of epitheliocytes

Group			An, μm	Pn, μm
Main	An, μm	Correlation coefficient	1	0.921**
		Value		0.001
		N	130	130
	Pn, μm	Correlation coefficient	0.921**	1
		Value	0.001	
		N	130	130
Comparison 1	An, μm	Correlation coefficient	1	0.329**
		Value		0.044
		N	38	38
	Pn, μm	Correlation coefficient	0.329**	1
		Value	0.044	
		N	38	38
Comparison	An, μm	Correlation coefficient	1	0.852**
		Value		0.001
		N	71	71
	Pn, μm	Correlation coefficient	0.852**	1
		Value	0.001	
		N	71	71

Note: ** p \leq 0.001

Conclusions

According to the study, it was found that more than 90.0% of patients with somatic pathology, especially if it is combined, have marked periodontium lesions according to the cytomorphometry data. Obviously, for COPD alone and in the more extent for COPD in combination with CHD mechanisms that stimulate the terminal stages of cell differentiation (maturation) are activated which is characterized by a decrease in the main formation, and leads to the development of destructive changes

Directions for future research

Further research opens the prospects for a more complete understanding of the ongoing disorders in the periodontium associated with an imbalance

in the periodontal complex. Epitheliocytes of the gum can change their functional state under the influence of exogenous and endogenous factors (somatic disease), including formation of chronic pathology. The increase in the nucleus is also associated with a decrease in the cell life span due to the action of somatic pathology. This factor is probably associated with a decrease in the production of young cells and the cellular composition is represented mainly by old and mature cells.

between aggressive and protective mechanisms, which would allow developing adequate measures to prevent dental morbidity in this category of patients.

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