МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ УКРАИНЫ
ХАРЬКОВСКИЙ НАЦИОНАЛЬНЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ

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МАТЕРИАЛЫ НАУЧНО-ПРАКТИЧЕСКОЙ КОНФЕРЕНЦИИ
С МЕЖДУНАРОДНЫМ УЧАСТИЕМ
«ГОФУНГОВСКИЕ ЧТЕНИЯ»
в рамках празднования 210-летия ХНМУ и международного Дня стоматолога

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Затверджений та рекомендований до видання Вченою радою Харківського національного медичного університету (протокол №1 від 22.01.2015 р.)

Збірка наукових праць присвячена 210-річчю Харківського національного медичного університету. У ній представлені матеріали науково-практичної конференції з міжнародною участю «Гофунговскі читання» у рамках святкування 210-річчя ХНМУ та міжнародного Дня стоматолога (10.02.2015 р.). Збірка включає останні результати наукових досліджень по актуальних проблемах стоматології та щелепно-лицьової хірургії з різних країн. У випуск включені праці фахівців, які виконані на кафедрах стоматологічного профілю та суміжних дисциплін медичних ВНЗ і установ післядипломної освіти лікарів, а також в практичній охороні здоров'я. У них відбиті експериментальні, теоретичні і клінічні питання сучасної стоматології та щелепно-лицьової хірургії. Представлені роботи з питань профілактики, діагностики, лікування і реабілітації стоматологічних захворювань у дорослих і дітей; педагогіки, історії стоматології, медичного краєзнавства та огляди літератури.

Автори виражають подяку за допомогу в публікації збірки Харківський обласний осередок Асоціації стоматологів України (голова осередку – кандидат наук з держ. управління, доцент Н.М. Удовиченко)

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Conclusions. With reference to the foregoing it is possible to draw a conclusion that CGP as well as conjoint course of CGP and lichen acuminatus are accompanied by significant changes of local immunity of the oral cavity which become apparent in the form of sharp decrease of lysozyme and beta-lysins activity, reduction of the amount of C3 components of complement and increase SIgA level in the oral fluid.

Efficiency of our method of treatment of patients with CGP associated with lichen acuminatus is proved through recovery of indices of local non-specific immunity of the oral cavity such as lysozyme and beta-lysins activity and concentration of C3 fragments of complement as well as normalization of SIgA level directly after the course is finished and in 3 months after treatment.

Normalization of indices of local immunity of the oral cavity is accompanied by absence of symptoms of inflammation of parodontium tissue.

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IMMUNOHISTOCHEMICAL STUDY OF P53 AND KI-67 EXPRESSION IN GINGIVAL

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**Background.** It has long been postulated an association between periodontitis and atherosclerosis and it’s outcomes.

**Objectives.** The objective of this study was to analyze the expression of Ki-67 and p53 in gingival epithelium.

**Material and Methods.** 29 pieces of periodontal tissues of patients who had died in Sumy Regional Hospital were evaluated for revealing Ki-67 and p53 by immunohistochemistry.

**Results.** Patients who had died from complications of atherosclerosis by immunohistochemistry basal cells layer were positive 93.2±1.9% \((P<0.05)\) for Ki-67, whereas only 50.7±0.5% of their gingival were positive for p53 \((P<0.05)\). With respect to the immunoexpression of Ki-67 in gingival of not atherosclerotic patients they had 39.85±2.77% \((P<0.01)\) of positive cells. In the gingival of not atherosclerotic patients presence of p53 was positive 2.07±0.6% \((P<0.05)\).

**Conclusions.** Proliferative gene Ki-67 expression among patients with not atherosclerotic conditions was low. Patients, who had died from complications of atherosclerosis with cellular hyperplasia, since it is merely an adaptive process, indicated an increased Ki-67 Gene and p53 expression. Among patients with atherosclerosis it is linked to cell’s arrests in the G1 phase of the cell cycle and that provides time for repair of the damaged DNA before entry into S or induction of apoptosis.

**Introduction.** Antigen Ki - 67 is the prototypic cell cycle-related nuclear protein, expressed in proliferating cells in all phases of the active cell cycle \((G1\rightarrow S\rightarrow G2\rightarrow M\text{phase})\) and reaches its peak in the G2 and M phases. It rapidly degrades after mitosis with a half life of detectable antigen being an hour or less. It is absent in resting \((G0)\) cells. Antibodies of Ki - 67 are useful in the cell growing fraction in neoplasms [1] Antigen Ki-67 expression also appears when DNA synthesis is stopped or when the cell undergoes apoptosis. However, according to other investigators, the markers of proliferation that are in use recently, such as Ki-67, can only
yield a limited picture of its involvement in the cell cycle [2]. Increase in Ki-67 expression was recently demonstrated in the gingival epithelial cells and fibroblasts of the lamina propria of patients with gingival overgrowth induced by nifedipine, phenytoin or cyclosporin A [3, 4]. Nevertheless, Ki-67 can be used to measure the growth fraction, both in normal, premalignant, and malignant tissues. [5] Some are also of the opinion that Ki-67 as tumor growth stimulating genes marker and p53 as tumor suppressor gene marker are closely associated with their prognostic value. Saito et al. found p53 expression in isolated cells of the suprabasal layer of the gingival epithelium in seven out of 11 patients with nifedipine-induced gingival enlargement [3].

Immunohistochemical markers, such as p53, and Ki-67, can be used in paraffin sections to show the presence of proliferative genes or cellular proliferation capacity and possible early malignant changes [6]. The study shows such an expression of p53, and Ki-67 in proliferative genes under the influence of atherosclerosis.

Methods. The study samples consisted of periodontal tissues of patients who died in Sumy Regional Hospital Patients and were investigated for p53 and Ki-67 antibodies. The subjects were divided into two equal groups: Patient’s Group (Group I): included 20 people who had died from complications of atherosclerosis. Control Group (Group II): included 9 patients with various diagnoses (not atherosclerotic ones)

Ethics statement. All study and family the deceased participants were informed about the study and those who agreed to participate signed a consent form. Ethical approval for conducting the study was obtained from the Health Ministry of Ukraine (HMU). Approval for performing oral examination was obtained from the authorities of the respective hospital and clinic. Information about the biopsy made in medical records and postmortem epicrisis Patients who agreed to take part in a surveillance confidential study, in accordance to the Local Research Ethics committee from medical institute of Sumy State University, Sumy Ukraine, approved under the number 013U003315, were enrolled in this study.

Paraffin sections were prepared for acridine orange staining by mounting on the slides, drying on a hot plate, and then sections were immersed into three sets of xylene for 2 minutes each followed by three sets of absolute ethanol for 5 minutes and finally rinsed with tap water. The aim was to remove the wax and dehydrate the sections. Slides (paraffin) were placed into acridine orange staining solution for 15 minutes, and rinsed with phosphate-buffered saline (PBS). Then the slide was soaked in 0.1% calcium chloride solution for 3 minutes and was washed with PBS once again. Cover glass was mounted for observation under a fluorescent microscope to observe and read the result.

Immunostaining for p53 and Ki-67 was performed on formalin-fixed (pH 7.4), paraffin-embedded thyroid tissue sections using mouse monoclonal antigens - p53 and Ki-67 (Thermo Fisher Scientific UK). Briefly, 4μm thick tissue sections were dewaxed in xylene and were brought to water through graded alcohols. Antigen retrieval was performed by microwaving slides in 10mM citrate buffer (pH 6.2) for 30 min at high power, according to the manufacturer’s instructions. To remove the endogenous peroxidase activity, sections were then treated with freshly prepared 1.0% hydrogen peroxide in the dark environment for 30 min at temperature 37°C. Non-specific antibody binding was blocked by dint of blocking serum. The sections were incubated at temperature 37°C for
30 minutes, with primary antibodies against p53 and Ki-67 diluted with ratio 1:100 in phosphate buffered saline (PBS) pH 7.2, after 3 washings with PBS. Anti-(mouse IgG)–horseradish peroxidase conjugate (1:40 000 dilution) was used for the detection of the p53 and Ki-67 primary antibodies, sections were then incubated for 20 min, at temperature 37° C. The color was developed by DAB.

Appearance of positive factors was detected semi-quantitatively by counting positive cells in visual field (p53, and Ki-67 – 0-5% - few, 5-20% - moderate, 20-40% - numerous, 40-100% - abundance of positive structures in visual field)

Data were analyzed using the program Origin Version 8. The Student method was used to perform simple comparative analysis. The variables were regarded as normally distributed.

**Results.** In Group I p53 and Ki-67 were expressed in all specimens. Expression of Ki-67 in gingiva is shown in figures 1 and 2. The immunoeexpression of these proteins was confirmed by the presence of brown stained nucleus in gingival. Ki-67 was expressed in gingival and stromal cells at the invasion front in all. In general, Ki-67 staining was more intense in the basal cells gingiva than in the stroma. By immunohistochemistry basal cells layer, 93.2±1.9% (P<0.05) of gingiva were positive for Ki-67 (Fig 1A), whereas only 50.72±0.5% of gingival were positive for p53 (P<0.05) (Fig 4A). In Figure 1, Ki-67 was expressed with apoptosis calves (Fig 1 C) of a score of 2 in cases of atherosclerosis. In Figure 2 we can see the direction epithelial proliferation (Fig 2 B) into inflammatory (Fig 2 A)
With respect to the immunoe-expression of Ki-67 in gingival group II, were detected 39.85±2.77% ($P<0.01$) of positive cells (Fig 3). In the gingiva immunoeexpression of p53 was classified with a score of 9 in most cases 2.07±0.6% ($P<0.05$) (Fig 5).

Induction of the enzymatic activity of Ki-67 was increased by p53. This could be explained by the fact that Ki-67 is physiologically expressed by the gingival and p53 expression is weak or absent in gingival and induced apoptosis in the granular layer of the epithelium.

**Discussion.** A study by Varga and Tyldeley, [7] have shown that squamous cell carcinoma may arise in gingival hyperplasia induced by cyclosporine. In the present group I of patients, the density of Ki-67-positive cells per mm$^2$ was significantly higher than among the patients of group II. However the p53 protein is arresting cells in the G1 phase of the cell cycle and allows time for repair of the damaged DNA before entry into S phase [8]. In normal tissues without impaired DNA, the half-life of protein p53 is too short to permit immunohistochemical detection. Nevertheless, antigen-retrieval techniques have resulted in the increased detection of p53 protein in normal tissues and it is possible
to immunohistochemically detect some p53-positive cells. This same effect is shown on patient’s gingival in the group I. Our study supports the Ki-67 protein expression as an absolute requirement for progressive growth of cell division [9].

Conclusions. Proliferative gene Ki-67 expression among patients with not atherosclerotic conditions was low. Patients, who had died from complications of atherosclerosis with cellular hyperplasia, since it is merely an adaptive process, indicated an increased Ki-67 Gene and p53 expression. Among patients with atherosclerosis it is linked to cell’s arrests in the G1 phase of the cell cycle and that provides time for repair of the damaged DNA before entry into S or induction of apoptosis.

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CORRECTION OF CYTOKINE MISBALANCE IN PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS OF INITIAL-I DEGREES OF SEVERITY BY MEANS OF LOCAL APPLICATION OF QUERCETINUM GRANULES AND LIPOSOMAL QUERCETINUM-LECITHIN COMPLEX
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Introduction. Efficiency of local application of medical drugs in periodontal tissues depends on the display of substances in the periodontal pocket (PP), choice of medical substances, method of his application, contact with the gingival oral mucosa and maintainance of this concentration. Therefore it is necessary advantage to give to the forms and pathways of medications with the controlled and long action [5, 6]. Development and application high-efficiency and safe facilities of drug therapy of chronic generalized periodontitis (HGP) the last years legally considered one of priority directions of native and foreign researchers [1, 2, 3]. Medical local therapy is inalienable part of complex treatment of HGP [4].

Liposomes, owing to their small size, penetrate regions that may be inaccessible to other delivery systems. It is noteworthy that only liposomes have been largely exploited for drug delivery because the methods of preparation are generally simple and easy to scale-up. The aim of using liposomal carriers is generally, to increase the specificity towards cells or tissues, to improve the bioavailability of drugs by increasing their diffusion through biological membranes, to protect them against enzyme in-