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Change of the cytokine profile parameters in oral fluid of patients with the generalized periodontitis under the influence of complex treatment using the method of photodynamic therapy and natural origin medicines

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Abstract
Twenty-eight patients were examined, aged from 20 to 44 years with no somatic pathology, including 8 persons with healthy periodontium and 20 patients with the chronic generalized periodontitis, where half of the people were diagnosed with the initial-I stage, while the rest had the I-II stage of the disease development. Content of the cytokines in oral fluid was estimated using the method of enzyme-linked immunosorbent assay before and after the treatment. Level of the proinflammatory cytokines IL-1β and IL-6 increased significantly in patients with the GP, while apparently the content of anti-inflammatory cytokine IL-10 decreased in oral fluid, especially in case of the GP at the I-II stage of development.
Complex treatment included professional hygiene, local and general medical treatment with natural origin medicines, as well as photodynamic therapy. As a result of combined therapy, patients with GP achieved significant normalization of level of the studied cytokines in oral fluid, especially in case of the GP at the initial-I stage: decreased IL-1β and IL-6 and increased IL-10 (with achieving health indicator), which is illustrative of the regulation of cytokine imbalance in local immune defense of oral cavity. The data received give us an opportunity to recommend the method we have developed for the GP treatment highly.

Keywords: Generalized periodontitis, oral fluid, interleukins, photodynamic therapy, natural origin medicines

1. Introduction
The state of local protective systems in oral cavity, including the cytokine profile of oral fluid is among many factors that cause a possibility of development and determine a progress of the generalized periodontitis (GP) [1]. It is known that the chronization of inflammation process in the periodontium is accompanied by changes in the cytokine system, the imbalance of which could disrupt the existing connections in the periodontal complex, causing the osteoporosis of alveolar bone, worsening the pathological process, secondary immunodeficiency, autoimmune diseases [2, 3]. This ultimately leads to the regeneration pathology, that is, the incomplete maturation of granulation tissue [4].
It has been established that some bacteria use host's cytokines as growth factors. Another mechanism for bacteria control over the complex of host's cytokines is a secretion of proteases. In particular, synthesis of such proinflammatory cytokines as IL-1β, IL-6, IL-8 is conducted by monocytes, pre-activated lipopolysaccharides of the bacteria. Periodontopathogens are modulating the cytokine network in such a way [5].
IЛ-1β has one of the most aggressive and damaging influences among the pro-inflammatory cytokines over the periodontium tissues [2]. IL-6 is a synergist to IL-1β; it activates the proliferation of T- and B-cells, increases the production of immunoglobulins, can enhance apoptosis, phagocytosis and lysozyme production [6].
Anti-inflammatory cytokines are the antagonists of the proinflammatory ones; they include IL-4, IL-10, TGF-β, etc. and have an anti-osteoporotic effect, as well as the ability to oppress the destructive inflammation process in the periodontal tissues [7]. IL-10 is one of the most important regulatory cytokines, which suppresses the cellular immune response and stimulates the humoral response instead [8]. It may be no accident that it received the characteristic of the factor that suppresses the synthesis of cytokines as its suppressive effect on production of the most proinflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α, G-CSF, etc.) has been proved [9].
Photodynamic therapy is one of the methods of effective influence on pathogenic flora and

microcirculation, on vasomotor abnormalities and on acceleration of tissue regenerating times [10]. It helps to reduce the number of pathogens more than in 92 % and preserves the composition of normal microflora, resulting in restoration of physiological equation between the aerobic and anaerobic microflora in oral cavity by a ratio of about 75 % / 25 % [11], which contributes to the normalization of local immunity too. This method should be used in periodontology in combination with medicines pathogenesis directed action.

IL-1β was extensively studied in patients with GP, unlike IL-6 and IL-8. Therefore, examining their content in patients with the chronic GP at the various stages of development, as well as examining the changes in their level under the influence of complex treatment are of scholarly interest, especially in connection with the photodynamic therapy, which is still little used in the periodontology.

Objective of the research was examining the influence of the complex method that was offered by us for the chronic GP treatment at the initial-I and I-II stage of development using the method of photodynamic therapy and natural origin medicines on the content of pro- and anti-inflammatory cytokines IL-1β, IL-6, and IL-10 in oral fluid.

2. Materials and methods

Twenty-eight patients were examined, aged from 20 to 44 years, with no with no somatic pathology, including 8 persons with healthy periodontium and 20 patients with the chronic generalized periodontitis, where half of the people were diagnosed with the initial-I stage, while the rest had the I-II stage of the disease development.

In order to establish a local immune status, levels of pro- (IL-1β, IL-6) and anti-inflammatory (IL-10) cytokines were examined, also the ratio of proinflammatory cytokines to anti-inflammatory was estimated (index rating), namely: 1β/IL-10 and IL-6/IL-10 index in oral fluid (which was taken in fasting state before and after the treatment into plastic toss-away tubes), using the method of the enzyme-linked immunosorbent assay on the Stat Fax 303 Plus with the help of reagents of the ZAO Vector-Best (Russia).

Professional hygiene was provided to all patients, local traumatic factors were eliminated, including the components of traumatic occlusion. Root planning was performed using the ultrasonic diamond-impregnated tips in the perio mode with final polishing by the Vector system. After the end of local manual treatment, the photodynamic therapy of periodontal pockets was conducted: one time in case of GP at the initial-I stage, 2 times – at the I-II stage. The GRANUM Diode Laser (manufactured by Spectrum International, Inc., USA) was used for this purpose. The Photolon photo synthetase was injected using the syringe with atraumatic needle into a periodontal pocket for 5 minutes. Then, the periodontal pockets were washed with distilled water and the gingivae were dried with the dry instrument. Afterwards, the light guide of the laser was inserted into the periodontal pocket using the following parameters: wavelength - 660 nm, mode – continuous, power – 200 mW, exposure time – 1-4 min and the pocket was processed by the cyclical motions across all its depth. After the procedure was completed, the periodontal pocket was washed with distilled water.

At the same time, the „Gengigel" gel based on the hyaluronic acid was used for local medical treatment by applying it on the gingivae twice per day for 20 min; treatment course is 10-12 days. For general medical treatment, the sublingual tablets „Apilak" were prescribed: 1 tablet 3 times a day; treatment course is 10-15 days.

Parametric methods of descriptive statistics were used while processing the data received with help of the "Statistika 6.0" Package for Microsoft Excel 2010.

3. Research results and their discussion

Significant increasing of the level of proinflammatory cytokines was observed in oral fluid of patients with the chronic GP (see table). Thus, the index of IL-1β content in case of GP at the initial-I stage compared with the healthy persons increased in 1.76 times ($p<0.001$), at the I-II stage – in 1.96 times ($p<0.001$); the difference between the groups was 11.3 % ($p_1<0.01$). Under influence of the treatment, concentration of IL-1β in patients at the initial-I stage decreased in 1.5 times ($p_2<0.001$) and at the I-II stage – in 1.54 times ($p_2<0.001$).

Amount of another proinflammatory cytokine – IL-6 – increased significantly too: at the initial-I stage – in 4.79 times ($p<0.001$) and at the I-II stage – in 6.26 times ($p<0.001$). When comparing the data at the different stages of GP, it has been established that in case of the greater stage of the disease, the number of IL-6 in oral fluid was 30.66 % bigger ($p_1>0.05$). Complex therapy helped to achieve a significant reduction of this index: in case of GP at the initial-I stage – to 64.81% ($p_2<0.05$) and at the I-II degree – to 54.11% ($p_2<0.05$).

Against the background of the increasing concentration of proinflammatory cytokines IL-1β and IL-6, the level of anti-inflammatory cytokine IL-10 decreased: in patients at the initial-I stage – in 1.68 times ($p<0.05$) and at the I-II stage – in 1.96 times ($p=0.001$). However, in case of GP at the I-II stage compared to the GP at the initial stage, number of IL-10 was 22.69% higher ($p_1>0.05$). The treatment we conducted had a positive impact on this index too: in patients at the initial-I stage, it increased by 69.86 % ($p_2<0.05$) and at the I-II stage – by 84.87 % ($p_2=0.001$), achieving the data of healthy persons ($p>0,05$).

IL-1β/IL-10 index in healthy persons was equal to 119.99; this ratio grew in 2.95 times in patients with GP at the initial-I stage and in 4.03 times in case of the I-II stage (fig. 1). Difference between the groups of patients in terms of IL-1β/IL-10 was 36.55 %. This index decreased after the treatment: initial-I stage – in 2.55 times, I-II stage – in 2.84 times.

While estimating the index of IL-6/IL-10 in patients with the intact periodontium, it was found that it was 0.4; in patients with GP at the initial-I stage, the index was 8.08 times bigger and at the I-II stage –12.98 times bigger (fig. 2). By comparing the index IL-6/IL-10 in both groups, it was established that patients with GP at the I-II stage had it in 60.19 % bigger. As a result of the complex therapy, this index decreased: in patients at the initial-I stage – in 1.16 times and at the I-II stage – in 1.82 times.

Index that was received as a result of examining the number of cytokines, correlated with the clinical picture of the disease.

Data that we received regarding the imbalance in case of GP between the proinflammatory (anti-osteoporotic) and pro-inflammatory (resorptive) cytokines towards the latter do not conflict with the most scientific research [2], but only confirm activation of the bone resorption in patients with GP, as well as starting the cascade of inflammation processes, which is closely connected with the abnormalities in the local immunoresistant state. As it was established that the

proinflammatory cytokines TNF-α, IL-8 and especially IL-1β contribute to the degradation of extracellular matrix, initiate the resorption, mediated by osteoblasts [5], and IL-1 induces production of collagenase in fibroblasts and inhibits the formation of collagen and bone tissue [12].

As a result of the complex treatment of patients with GP, where the main emphasis was put on the photodynamic therapy of periodontal pockets, there was a positive dynamics of interleukins indices, the values of which were close to be normal. It proves that we have developed the method of treatment, which has an immune-modulating effect either by means of local effect of photodynamic therapy on the pathogenic flora of periodontal pockets or thanks to the exogenic effect of the „Gengigel" gel and the endogenous effect of the „Apilak".

Locally acting product that we used called the „Gengigel" was designed based on the hyaluronic acid, which has the ability to stimulate reparative processes in a body, provides numerous features of connective tissue (trophic, barrier, plastic) through the active exchange of substances between blood and tissues by stabilizing the intercellular substance, prevents penetration of microorganisms, viruses, and toxins in the periodontium tissues, and is directly involved in the processes of inflammation and immunity [13].

The „Apilak" preparation that was used for general medical treatment is the drug of natural origin and was designed based on the bee royal jelly, which affects both the processes of regulation and metabolism of cells and tissues, by restoring the trophic processes at the cellular level, stimulates phagocytosis, and reduces the processes of free radical oxidation in a body [14, 15].

Therefore, all the above-described effects of the drugs that were used as a complex, combined with the photodynamic therapy, helped to achieve normalization of the local immune condition of oral cavity.

Table 1: Dynamics of cytokines level in oral fluid of patients with the chronic generalized periodontitis under the influence of complex treatment (M±m)

Index, terms of research	Healthy persons, n=8	Patients with the chronic GP	
		initial-I stage, n=10	I-II stage, n=10
IL-1β, pg/ml before the treatment	293,98±15,71	517,23±24,39 p<0,001	575,67±35,45 p<0,001 p_1>0,05
after the treatment		343,89±23,93 p>0,05 p_2<0,001	374,15±33,86 p>0,05 p_1>0,05 p_2=0,001
Δ, abs. (%)		-173,34 (50,41%)	-201,52 (53,86%)
IL-6, pg/ml before the treatment	0,988±0,12	4,73±0,58 p<0,001	6,18±0,64 p<0,001 p_1>0,05
after the treatment		2,87±0,49 p<0,01 p_2<0,05	4,01±0,58 p=0,001 p_1>0,05 p_2<0,05
Δ, abs. (%)		-1,86 (64,81%)	-2,17 (54,11%)
IL-10, pg/ml before the treatment	2,45±0,25	1,46±0,25 p<0,05	1,19±0,18 p=0,001 p_1>0,05
after the treatment		2,48±0,26 p>0,05 p_2<0,05	2,20±0,18 p>0,05 p_1>0,05 p_2=0,001
Δ, abs. (%)		-1,02 (69,86%)	-1,01 (84,87%)

Note. Possibility of different values is indicated: p – to a value in healthy persons; p_1 – to a value in patients with GP at the initial-I stage; p_2 – to a value in patients with GP before the treatment.

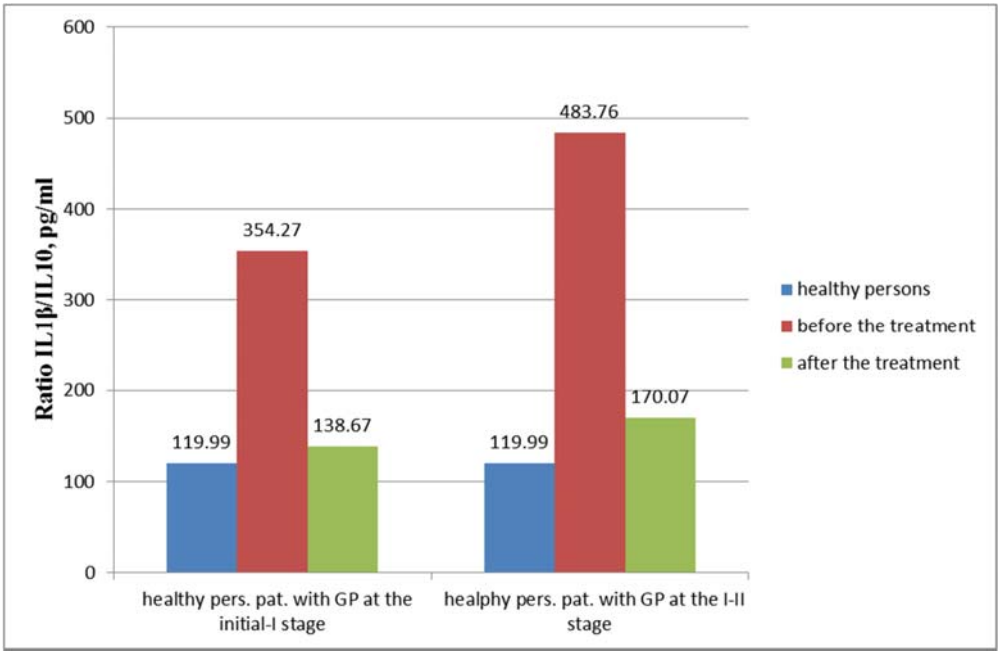

Fig 1: Changes of the IL1β/IL10 ratio in patients with GP under the influence of complex treatment

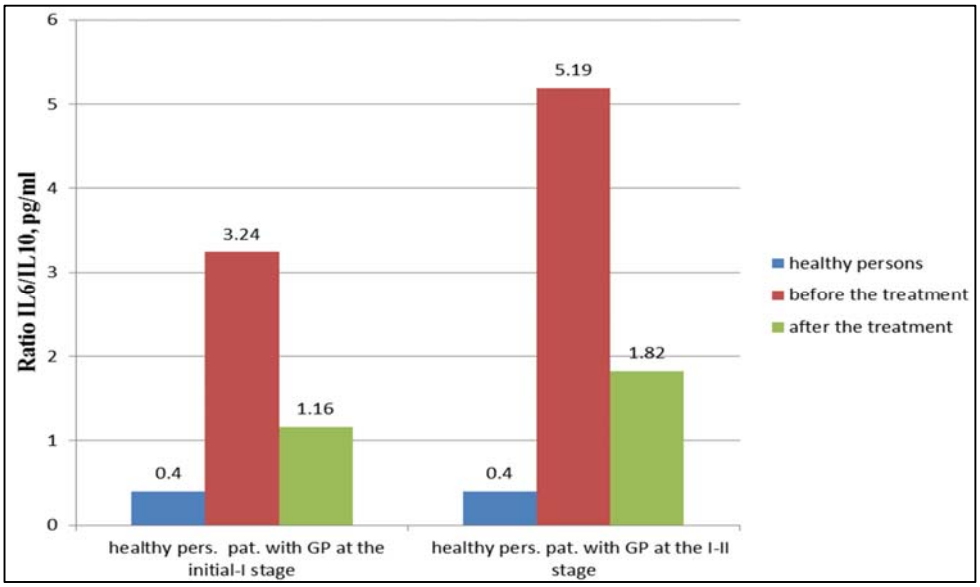

Fig 2: Changes of the IL6/IL10 ratio in patients with GP under the influence of complex treatment

4. Conclusions

1. Content characteristics of the proinflammatory cytokines IL-1β and IL-6 increased significantly in patients with GP at the initial-I and I-II stage, while the level of anti-inflammatory cytokine IL-10 was decreasing in oral fluid; these deviations were more substantial in case of GP at the I-II stage of development.
2. As the result of complex treatment with the use of photodynamic therapy, natural origin medicines (Apilak" and Gengigel") the substantial normalization of levels of the examined cytokines in oral fluid was achieved, especially in case of GP at the initial-I stage as follows: reduced IL-1β and IL-6 and increased IL-10 (with achieving health indicator), which is illustrative of the regulation of cytokine imbalance in the local immune defense of oral cavity.
3. The received data give us an opportunity to recommend the method we have developed for the GP treatment highly.

Follow-up study of the complex GP treatment is the direction for future research.

5. Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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