**Evaluation of prognostic significance of nitrite and cytokine profile (TNF-α) content in young people (18-25 years) in the development of periodontal tissue diseases**

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**Abstract**

Immunological changes in periodontitis are characterized by disturbances in the interaction of factors of nonspecific resistance of the organism, inhibition of cellular and humoral immunity, as well as inhibition of a relatively autonomous system of local immunity with an imbalance of nitrite and cytokine indices.

Assessment of a patient with periodontitis in modern conditions involves a comprehensive examination, which includes, in addition to traditional clinical methods, microbiological and immunological studies that allow objectifying the patient's condition, predicting the course of the disease and analyzing the effectiveness of therapeutic measures, which formed the aim of the study: to determine the prognostic significance of nitrite and cytokine profile (TNF-α) content in young people (18-25 years) in the occurrence and development of periodontal tissue diseases.

80 young people (18–25 years old) were under observation, among whom 37 people were diagnosed with early initial periodontitis, stage I (group I), 22 patients with catarrhal gingivitis (group II), and 21 patient with intact periodontium (group III).

 According to the results of the survey, a part of young people was found to have a bad habit – smoking. It was of interest to trace the influence of this harmful habit on the level of TNF-α cytokine and the amount of nitrites in the oral fluid. It was found that determination of nitrite content and proinflammatory cytokine-TNF-α in the oral fluid of young people (18-25 years old) has prognostic significance in the initiation and development of periodontal tissue diseases.

**Key words:** TNF-α, cytokine, nitrite, smoking, young people (18-25 years), generalized periodontitis, chronic catarrhal gingivitis

**Background**

The high prevalence of periodontal disease among students, a variety of local and systemic risk factors in the development of this pathology, the lack of an effective program of primary and secondary prevention of dental diseases in young people (aged 18–25) is currently one of the leading problems in therapeutic dentistry [1]. Immunological changes in periodontitis are characterized by disturbances in the interaction of factors of nonspecific resistance of the organism, inhibition of cellular and humoral immunity, as well as inhibition of a relatively autonomous system of local immunity with an imbalance of nitrite and cytokine indices [2].

Cytokine imbalance clearly correlates with the severity of pathology in patients with periodontitis, with a significant increase in the level of such pro-inflammatory cytokine as TNF-α [3]. TNF-α (also called cachectin) is a pyrogen, largely duplicating the effect of IL-1, but also playing an important role in the pathogenesis of septic shock caused by gram-negative bacteria. Under the influence of TNF-α, the formation of hydrogen peroxide and other free radicals by macrophages and neutrophils sharply increases. In chronic inflammation, TNF-α activates catabolic processes and thereby contributes to the development of cachexia, a symptom of many chronic diseases [4].

TNF-α is considered as the main mediator that determines the development and progression of inflammation in periodontal tissues [5]. There is evidence that with the pathology of periodontal tissues, it can be detected in the gingival fluid even before clinically significant manifestations of the disease and thus serve as its indicator [6]. Probably, an increase of TNF-α in inflammatory and destructive periodontal processes is protective in relation to the microflora invading its tissue. It is known that TNF-α has an inhibitory effect on the growth of staphylococci and has the ability to neutralize bacterial toxins in gram-negative infections. A direct relationship has been established between the presence of Porphyromonas gingivalis in the oral cavity of individuals with periodontal pathology and the expression of TNF-α in its tissues [7]. However, in addition to the protective function against invasion of microorganisms, TNF-α also performs a destructive role in relation to tissue structures. It occupies a key position in the pathogenesis of inflammatory-induced bone loss during periodontitis [8].

Also, nitric oxide, which is produced under the influence of iNOS, primarily has a cytotoxic and cytostatic effect on the pathogenic microflora. However, in addition to the protective effect, an excess of nitric oxide during inflammation (production of peroxynitrite) leads to pathological changes, which are based on the modification of macromolecules of proteins and lipids, inhibition of cell growth and reproduction [9]. In periodontitis, the rate of the nitrate reductase reaction and the nitrite content changes; In addition, an increase in the activity of hyaluronidase and b-glucuronidase is characteristic. The intensity of peroxidase processes in saliva increases by 1.5 times, and the content of lysozyme falls by 20-40%. Changes in defense systems are combined with an increase in the number of thiocyanates by a factor of 2-3 [10].

Nitrates (NO3-) and nitrites (NO2-) enter the saliva with food, tobacco smoke and water. With the participation of nitrate reductase bacteria, nitrates are converted into nitrites and their content depends on smoking. It has been shown that smokers and people engaged in tobacco production develop leukoplakia of the oral mucosa, and the activity of nitrate reductase and the amount of nitrites increase in saliva. The formed nitrites, in turn, can react with secondary amines (amino acids, drugs) to form carcinogenic nitroso compounds. This reaction takes place in an acidic environment, and it is accelerated by thiocyanates added to the reaction, the amount of which in saliva also increases during smoking [11]. In addition, excessive production of nitric oxide leads to persistent dilation of blood vessels, impaired metabolic processes, increased vascular permeability and, as a result, tissue edema [9]. The role of NO in the pathogenesis of GP has been actively studied for a number of years, since microcirculatory disorders and endothelial dysfunction are the most important components of its development [12].

Assessment of a patient with periodontitis in modern conditions involves a comprehensive examination, which includes, in addition to traditional clinical methods, microbiological and immunological studies that allow objectifying the patient's condition, predicting the course of the disease and analyzing the effectiveness of therapeutic measures [2], which formed the aim of the present study: to determine the prognostic significance of nitrite and cytokine profile (TNF-α) content in young people (18-25 years) in the occurrence and development of periodontal tissue diseases.

**Materials and methods**

80 young people (18–25 years old) were under observation, among whom 37 people were diagnosed with early initial periodontitis, stage I (group I), 22 patients with catarrhal gingivitis (group II), and 21 patient with intact periodontium (group III). The periodontal status of all examined was evaluated on the basis of traditional objective periodontal indices and clinical symptoms: papillary-marginal-alveolar index - PMA, size of periodontal pocket (PP), level of epithelial attachment loss (EAL), degree of gingival recession, abnormal mobility of teeth, severity of gingival bleeding , the presence of exudation from PP. Hygienic condition was assessed by the OHI-S index. The diagnosis of periodontal diseases was made in accordance with the classification of periodontal and peri-implant diseases and conditions (Chicago, 2017) [13].

In order to obtain additional information on the presence of local risk factors, a questionnaire was proposed, which each survey participant filled out, along with informed consent to participate in the study.

Biochemical analysis of oral fluid was based on a method based on a three-stage sandwich variant of solid-phase ELISA. The study was carried out using ready-made reagents from the kit for the determination of TNF-α (Vector-Best, Russia) using the automatic analyzer LabLine-100 (calibration certificate No. 37/1779 of 06/14/2016). In the first stage, all types of samples were incubated in wells with immobilized antibodies. Available TNF-α in the samples was associated with antibodies. The next stage was incubation with conjugate No. 1. In the third stage, the complex reacted with conjugate No. 2. All unrelated components were removed by washing after the appropriate steps. The amount of bound conjugate No. 2 was determined by the reaction with the use of peroxidase substrate chronum - hydrogen peroxide and the chromogen - tetramethyl benzene. The nitrite content was determined by a photo-colorimetric method using Griess reagent (0.4 g / ml) and 70% ice-cold alcohol, and using an automatic LabLine-100 analyzer. Gryss reagent is a mixture of sulfanilic acid and α-naphthylamine, which do not interact without the presence of nitrites. Nitrites interact with primary amines to form diazonium salts. With their further combination with aromatic compounds containing amine and hydroxo groups, an azo dye is formed (proportional to the amount of nitrite ions), whose concentration is determined photometrically — the absorption maximum at a wavelength of 545 nm. Thus, the method was based on the diazotization of sulfanilic acid with nitrites, followed by the interaction of the formed diazonium salt with α-naphthylamine to form a red-violet dye. Salts of heavy metals and turbidity interfered the determination, therefore, freezing was performed to precipitate the proteins present in the samples, after centrifugation was used to determine only supernatant.

 Statistical processing of the obtained results was carried out using the Microsoft Office Excel application package, as well as using the MedCalc program. For the correlation analysis, the Spearman correlation coefficient was used.

**Results of the study**

Analysis of the results of the periodontal examination of young people (18-25 years old) of the three groups showed that when distributed by gender among the examined students of group I (GP), 29.73% were men and 70.27% - women, in group II (CG) 31.82% were men and 68.18% were women. Among group III students (persons with intact periodontium), women predominated - 71.43%, while men were only 28.57%. Thus, the highest prevalence of the initial symptoms of inflammatory damage to the periodontal tissues was found among females.

 When determining the level of cytokine - TNF-α in the oral fluid depending on gender, the following results were obtained: the average content of TNF-α in women with intact periodontium was 0.11 ± 0.04 pg/ml, and in men - 0.06 ± 0.03 pg/ml. In the group with CG, the average content of TNF-α in women was 0.20 ± 0.06 pg/ml, and in men - 0.19 ± 0.08 pg/ml. In the group with GP, the level of TNF-α was 0.96 ± 0.22 pg/ml and 1.09 ± 0.33 pg/ml in women and men, respectively. Based on the data obtained, it was found that in the oral fluid, the level of TNF-α was increased in groups with CG and GP compared with a group of individuals with intact periodontium in women two and nine times, respectively, and in men three and more than ten times , respectively.

 When determining the quantitative content of nitrites in the oral fluid of the examined three groups, followed by analysis depending on gender, it was found that the average nitrite content in women with intact periodontium was 12.05 ± 2.08 μmol/l, and in men - 11.5 ± 3.18 µmol/l; in CG group, the average content in women was 12.38 ± 3.01 μmol/l, and in men - 16.28 ± 5.85 μmol/l; in group with GP, the average content in women was 13.5 ± 3.22 μmol/l, and in men - 16.12 ± 5.82 μmol/l. Consequently, the quantitative content of nitrites in women in groups with CG and GP compared with the group of individuals with intact periodontium did not differ much, while among men in groups with CG and GP compared with the group of persons with intact periodontium it increased 1.5 times.

 According to the results of the survey, a part of the surveyed young people (18-25 years old) was found to have a bad habit - smoking (Figure 1).

When comparing the data of groups I and II, it was found that bad habit — tobacco smoking was significantly more common in the examined group I (p = 0.004). Similar results were obtained when comparing groups I and III (p = 0.046). Thus, on the basis of an analysis of the results of the survey, it was revealed that a harmful habit — smoking was most common in persons with diagnosed GP.

In this regard, it was of interest to trace the influence of this harmful habit on the level of TNF-α cytokine and the amount of nitrites in the oral fluid (Tables 1, 2).

The influence of the harmful habit of smoking on the quantitative content of nitrites in the oral fluid of young people (Table. 2).

As a result of statistical analysis, significant differences were found between the data of content of TNF-α in smoking patients of groups I and III (p = 0.0025), and also between the data of smoking patients of groups I and II (p = 0.035). Similar, significant differences were found among non-smoking patients of groups I and III (p = 0.0033) and groups I and II (p = 0.0093).

In addition, significant differences were determined between the data of the quantitative content of nitrites in non-smoking patients of groups I and III (p = 0.014), and also between the data of patients of groups I and II (p = 0.034). It was found that in smokers with intact periodontium nitrite levels were reduced by half in relation to non-smokers, but in groups with CG and GP, on the contrary, the amount of nitrites in smokers in relation to non-smokers was increased by 1.5 and 3.5 times, respectively. Directly among smokers, there was a tendency to an increase in the amount of nitrite in the oral fluid in the groups with CG and GP in relation to persons with intact periodontium by 2.8 and 3.4 times, respectively.

The result of the correlation analysis depending on the prevalence in different groups (I-III) of the examined young people (18-25 years old) of the harmful habit - tobacco smoking was a statistically significant relationship of the average strength (r = 0.419; p = 0.0098) of TNF-α level only in patients of I group with diagnosed GP. This fact confirms the importance of the harmful habit of smoking in the generalization of inflammatory process in periodontal tissues.

**Conclusions:**

1. Among young people (18-25 years old), the highest prevalence (51.25%) of the initial symptoms of inflammatory damage to periodontal tissues (CG, GP) was found among females.

2. An increase in the oral fluid of both genders with diagnosed CG and initial periodontitis of level of pro-inflammatory cytokine TNF-α (in women 2 and 9 times, p=0.0003; in men 3 and more than 10 times, p=0.0015 ) confirms its role in the initiation of inflammatory changes in periodontal tissues.

3. The quantitative content of nitrites in the oral fluid of young people diagnosed with CG and GP among smokers in relation to non-smokers increased by 1.5 and 3.5 times, respectively. At the same time, among smokers, there was a tendency to an increase of nitrite content in the oral fluid among persons with CG and GP in relation to persons with intact periodontium by 2.8 and 3.4 times, respectively. Consequently, an increase of nitrite in the oral fluid reflects both the appearance of the first symptoms of inflammation in periodontal tissues and the negative effect of tobacco smoking on the development of the pathological process in periodontal tissues.

4. Among young people (18-25) with diagnosed GP, the most common was harmful habit - tobacco smoking (46%) with the highest content of pro-inflammatory cytokine TNF-α in the oral fluid (p <0.05). Established statistically significant relationship of average strength (r = 0.419; p = 0.0098) between the presence of a bad habit - tobacco smoking and the content of TNF-α in the oral fluid only in the group of persons with diagnosed GP, which confirms its importance in the generalization of inflammatory process in periodontal tissues.

5. Determination of nitrite content and proinflammatory cytokine-TNF-α in the oral fluid of young people (18-25 years old) has prognostic significance in the initiation and development of periodontal tissue diseases.

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