

ORIGINAL ARTICLE

Correction of the trace elements content in the oral liquid of patients with periodontitis influenced by complex non-surgical treatment

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ABSTRACT

Aim: To investigate the mineral homeostasis of oral liquid in patients affected by periodontitis and to analyze the impact of the implemented non-surgical therapy on the content of trace elements in the oral liquid at different observation periods, which depended on the severity of the disease.

Materials and Methods: There were somatically healthy individuals enrolled in this study, among them were 70 patients with periodontitis stage I and II, grade A and B and 17 individuals with intact periodontium. The content of Iron, Zinc, Copper and Manganese in oral liquid was determined by adsorption spectrophotometer before, after and 6 and 12 months following the treatment. Comprehensive non-surgical treatment included exo- and endogenous therapy with the microalgae *Spirulina platensis*.

Results: Examination of all patients with periodontitis demonstrates the content of biometals in the oral liquid with a probably decrease of Iron, Zinc, Manganese and an increase of Copper ($p < 0.001$). Changes in the mineral composition depended on the stage and degree of severity and were intensified in the subgroup I B, and even more so in the subgroup II B. After complex treatment administered the amount of Copper and Manganese was normalized (at all terms, for both stages of the disease) and the content of Iron and Zinc was also regulated (especially 6 months after in the subgroups IA and IIA).

Conclusions: It was established that the method of complex treatment based on administration of the blue-green microalgae *Spirulina platensis* contributes to the normalization of trace element composition in the oral liquid of patients with periodontitis.

KEY WORDS: periodontitis, Iron, Zinc, Copper, Manganese, oral liquid

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INTRODUCTION

According to modern scientific researches, the leading role in the pathogenesis of chronic periodontitis belongs to the complex immune-inflammatory reactions of the macro-organism initiated by the plaque biofilm [1]. There are numerous exogenous and endogenous factors such as host genetic component, smoking and diet that can affect the host's immune-inflammatory response and to contribute to disease progression. The reason for the active alveolar bone resorption are processes accelerated by any inflammatory diseases, immunological damage to cell membranes, the influence of various stress factors on the body, carcinogenesis, lack of vitamins and some trace elements, radiation and aging [2].

The principal role of micro- and macro- elements is crucial for the physiological cell functioning, therefore their alterations in biological fluids can be related to the underlying pathological condition. Data of researchers show that oral liquid is a biological environment with a complex micro- and macro-elemental composition

and is a place of constant interaction of the oral cavity organs [3-4].

Deficiency of essential trace elements and accumulation of toxic ones in saliva have a local damaging effect and contribute to the formation of periodontal diseases. Periodontal tissues become susceptible to damaging agents of oxidative stress due to an imbalance of biometals in various biological environments. As a result the reparative activity is inhibited, metabolic and structural changes occur [5].

Natural agents with antimicrobial, antioxidant and anti-inflammatory properties deserve special attention in treatment of periodontal diseases. They act gently, have a regulating and normalizing effect, are easily absorbed and are non-toxic, have few adverse reactions such as the development of drug resistance, hypersensitivity and suppression or imbalance of the normal oral microflora [6].

In view of the above, the study of the biochemical composition in the oral liquid is important in the diag-

nosis of periodontal disorders as well as being helpful for the assessment of the complex treatment used.

AIM

To investigate the mineral homeostasis of oral liquid in patients affected by generalized periodontitis and to analyze the impact of the implemented non-surgical therapy on the content of trace elements at different observation periods, which depended on the severity of periodontitis.

MATERIALS AND METHODS

There were 87 somatically healthy 25-44-year-olds non-smokers enrolled in this study.

Among them, there were 70 patients with periodontitis stages I and II, grades A and B according to Classification of Periodontal and Peri-Implant Diseases and Conditions, 2017 [7], and 17 people with intact periodontium (to compare the indicators of affected patients and healthy individuals before therapy and during the treatment). Patients with periodontitis were further divided into I and II groups according to the stages of the severity, and into A and B subgroups according to the rate of progression. Group I included 26 people - 13 in each subgroups I A (stage I of severity, grade A slow rate of progression) and the subgroup I B (stage I of severity, moderate rate of progression), respectively. 44 persons were included in the group II - 21 in the subgroup II A (moderate stage of severity, moderate rate of progression), 23 in the subgroup II B (moderate stage of severity, moderate rate of progression).

The inclusion criteria for the study were: age 25-44 years; patients with periodontitis of stages I and II, grade of progression A and B, without any general somatic pathology, who were not recorded with any medical registration; also with no contraindications to administered treatment regarding pharmacological agents; non smokers; individuals who followed the doctor's recommendations and strictly adhered to the rules of the controlled study, and provided a signed consent permission.

The exclusion criteria were: patients with somatic pathology who were recorded with medical registration; who express some contraindications to recommended treatment and pharmacological agents; pregnancy and lactation or planning of pregnancy during the study; bad habits, such as: smoking, alcohol and drug addiction; untreated or complicated dental caries; orthodontic pathology; presence of irrational and traumatic orthopedic appliances; partial adentia (more than three

teeth); the oral mucosa pathologies; participation in other clinical studies; violation of the conditions of a clinical controlled study.

Healthy individuals were selected according to the same inclusion and exclusion criteria as patients with periodontitis, but an additional condition was the absence of periodontitis.

The level of Iron (Fe), Zinc (Zn), Copper (Cu) and Manganese (Mn) in the oral fluid was determined four times using a C-115 PC Atomic Adsorption Spectrophotometer: before, after, 6 and 12 months after therapy. Oral liquid of 10 ml was collected before breakfast in the morning between 8:00 a.m. to 10:00 a.m. The data of each patient at each stage of the study was recorded into a special examination card we developed. They became the material for statistical processing.

Complex non-surgical treatment consisted of exo- and endogenous therapy. The patients were prescribed with tablets of the blue-green microalgae *Spirulina platensis*, to be taken 1-2 g (depending on the severity) twice a day, 20 minutes before meals, for a course of 4 weeks. Local therapy comprised the initial periodontal therapy according to the generally accepted method, which depended on the clinical condition, the stage and degree of the disease. We used our extemporaneous paste, which contained equal parts of powdered blue-green microalgae "*Spirulina*" and powder of silica enterosorbent "*Sylard-P*", mixed in a 0.05% solution of Chlorhexidine bigluconate to a gel-like consistency for applications and instillations into periodontal pockets.

"*Sylard-P*" is a medical sorbent approved for clinical use by the Pharmaceutical Committee of Ukraine (protocol No. 1 dated January 23, 1993). The agent is created on the basis of highly dispersed silicon dioxide. Its specific surface is 300 mg per 1 g of substance. This property is responsible for its high adsorption capacity, 1 g of "*Sylard-P*" binds up to 300-800 mg per 1 g of protein substances and more than 10^9 microorganisms. The obtained mixture was introduced into periodontal pockets and applied to the gingival mucosa for 20-30 minutes. The course lasted for 6-8 procedures after 1-2 days. The microalgae agent "*Spirulina*" was prescribed by 1.0-2.0 g to be taken twice a day (or 1.0 g three times a day) for 4 weeks.

Chemical composition of "*Spirulina*" is: protein 60-70%; carbohydrates 10-20%; lipids- 5%. Vitamins (mg/kg): B-carotene (provitamin A) -1700; B₁-55; B₂-35; B₅-11; B₆-3; B₁₂-1.6; B₉-0.5; E-190; C-15; RR-118. Minerals and trace elements (mg / kg): Calcium -1180; Phosphorus - 8280; Iron - 528; Sodium - 344; Magnesium - 1663; Iodine - 3; Selenium - 2, etc.

This particular composition of the paste was chosen based on its multidirectional effect on periodontal tis-

sues such as antimicrobial, anti-inflammatory, sorption, antioxidant, as well as for normalizing microelement metabolism, maintaining the optimal level of redox biometals in periodontal tissues in order to reduce oxidative stress and to activate antioxidant processes in periodontal tissues. This helps to inhibit the destruction of connective tissue and alveolar bone. The action of the paste is also enhanced by the general effect of the spirulina prescribed to be taken orally.

Since the treatment of periodontitis requires staged constant therapy, the control, reassessment and local supportive treatment were conducted 6 months after according to each specific clinical situation, using an exogenous recommended by us treatment. If needed, the local treatment was administered again 12 months after, and for groups II A and II B – endogenous therapy as well.

The clinical study was conducted in accordance with the legislation of Ukraine and the principles of the Helsinki Declaration on Human Rights, without the participation of pharmaceutical companies. This study was approved by the Ethical Committee of Ivano-Frankivsk National Medical University (Protocol No 14/06 from 23/11/06). We followed the ethical standards when working with the examined and obtained the written permission from each participant for collection and investigation of biological materials.

Statistical processing of the gained results was carried out by a personal computer, using the Microsoft Excel spreadsheet program. Statistical management of materials was conducted with the "STATISTICA 6.0" package. Analysis of the distribution of the original data (measurement results of the studied indicators) obtained by the Shapiro-Wilk test showed a distribution close to normal. In view of this, to substantiate the hypothesis of equality (presence of difference) of mean values in unrelated (independent) and related (dependent) samples the paired and unpaired Student's t-tests were employed. The paired criterion was applied for dependent samples, namely: to assess the hypothesis that the mean values in the experimental groups (I and II), in particular in the subgroups (IA, IB, IIA and IIB) before and at different times after treatment, proved to be different. The unpaired Student's criterion, on the other hand, was used to check the hypothesis of a difference in data of independent samples, in particular between healthy individuals and all examined of IA and IB subgroups, as well as between healthy and examined of IIA and IIB subgroups. For correlation analysis, we used Spearman's rank correlation coefficient, which is an indicator of the monotonicity of the relationship between two indicators

AI STATEMENT

The author states that no AI tools were used in writing this article.

FRAMEWORK

The study was performed within the framework of the scientific topics "Differentiated approaches to the treatment and prevention of dental diseases in the population of Precarpathia" (state registration number 0121U111146) researched by the Department of Pediatric Dentistry of Ivano-Frankivsk National Medical University.

RESULTS

The study shows that there is an imbalance of biometals in the oral liquid of all examined patients affected by periodontitis. Thus, in subgroup I A the level of Fe was reduced in 1.16 times, and in subgroup I B in 1.23 times ($p1 < 0.001$) compared to healthy people (Table 1).

Complex treatment contributed to a significant increase of its content in subgroup I A - in 1.18 and in 1.17 times immediately, 6 and 12 months after ($p2 < 0.001$), with the achieved indicators being higher than in healthy people. Complete normalization of the Fe content in the oral fluid also occurred in subgroup I B ($p2 < 0.001$).

The level of Cu was also elevated in patients with chronic periodontitis in comparison to individuals with intact periodontium, but in the subgroup I A it was implausible ($p1 > 0.05$), and in I B - reliable ($p1 < 0.005$). Analysis demonstrates that after appointed therapy the concentration of Cu in the oral fluid of patients in subgroup I A has sharply decreased in 1.21 times ($p2 = 0.001$), exceeding the norm in 1.10 times ($p1 < 0.05$). Subsequently, there was a raise in the level of this trace element, and the difference with the data gained before treatment became insignificant ($p2 > 0.05$). According to the Cu index in the subgroup I A there was a significant difference ($p3 < 0.05$) found 6 and 12 months after compared to the data gained immediately after treatment, therefore the achieved result was not maintained in the long-term observation period.

In patients with periodontitis of subgroup IB the Cu content was higher in 1.18 times ($p1 < 0.005$) before treatment. It lowered immediately after its completion in 1.17 times ($p2 < 0.005$) and reached the data in healthy people. The amount of Cu fluctuated slightly, but remained at a level close to the normal values even in the long term of observation ($p2 < 0.05$).

The analysis of the data regarding the amount of Zn in the oral liquid showed that this indicator was reduced

Table 1. Changes of biometals in the oral liquid of patients with periodontitis stage I, grade A and B due the complex treatment (M±m)

Indicators	Healthy n=17	Subgroup I A				Subgroup I B			
		Before treatment, n=13	After treatment, n=13	6 months after, n=12	12 months after n=10	Before treatment, n=13	After tratment, n=12	6 moths after, n=10	12 months after, n=9
Fe, mcg/l	763.00± 21.14	658.47 ±14.60 p ₁ <0.001	779.19± 16.63 p ₁ >0.05 p ₂ <0.001	774.77± 16.66 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	769.90± 14.06 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	619.86± 28.14 p ₁ <0.001	763.67± 11.72 p ₁ >0.05 p ₂ <0.001	770.25± 19.00 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	755.34± 14.39 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05
Cu, mcg/l	80.25± 2.65	87.72 ±3.11 p ₁ >0.05	72.48± 2.38 p ₁ <0.05 p ₂ =0.001	81.34± 2.77 p ₁ >0.05 p ₂ >0.05 p ₃ <0.05	82.01± 2.58 p ₁ >0.05 p ₂ >0.05 p ₃ <0.05	94.61± 3.26 p ₁ <0.005	80.62± 2.88 p ₁ >0.05 p ₂ <0.005	83.34± 2.48 p ₁ >0.05 p ₂ <0.05 p ₃ >0.05	82.48± 2.82 p ₁ >0.05 p ₂ <0.05 p ₃ >0.05
Zn, mcg/l	521.59± 14.64	454.70 ±18.96 p ₁ =0.01	534.30± 14.28 p ₁ >0.05 p ₂ <0.005	529.99± 13.73 p ₁ >0.05 p ₂ <0.005 p ₃ >0.05	523.13± 12.68 p ₁ >0.05 p ₂ <0.01 p ₃ >0.05	440.06± 16.35 p ₁ =0.001	522.30± 14.64 p ₁ >0.05 p ₂ =0.001	513.98± 11.43 p ₁ >0.05 p ₂ =0.001 p ₃ >0.05	511.30± 12.95 p ₁ >0.05 p ₂ <0.005 p ₃ >0.05
Mn, mcg/l	26.16± 1.38	20.12 ±1.60 p ₁ <0.01	27.20± 1.32 p ₁ >0.05 p ₂ <0.005	26.26± 1.37 p ₁ >0.05 p ₂ <0.01 p ₃ >0.05	26.12± 1.45 p ₁ >0.05 p ₂ <0.05 p ₃ >0.05	16.88± 1.51 p ₁ <0.001	26.34± 1.22 p ₁ >0.05 p ₂ <0.001	26.11± 1.32 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	25.46± 1.27 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05

Note. Here and in Table 2 is indicated the probability of the difference: p₁ - to the value in healthy people; p₂ - to the value before treatment; p₃ - to the value immediately after treatment

Source: compiled by the authors of this study

in 1.15 times in patients with periodontitis in subgroup I A, and in subgroup I B in 1.19 times (p₁=0.01; p₁=0.001). It advanced above the norm in both subgroups immediately after treatment, and the difference with the data before treatment was 1.16 and 1.17 times (p₂<0.005; p₂=0.001). Six months and a year later, the Zn level remained higher in patients of subgroup I A than in healthy individuals, and in subgroup I B this indicator slightly decreased, but stayed close to the norm (p₂=0.001; p₂<0.005).

The content of Mn compared to healthy ones was reduced in 1.30 (p₁<0.01) and in 1.55 (p₁<0.001) times in subgroups I A and I B, respectively. Its level advanced due to complex therapy used and was regulated very similarly to changes of Zn over all observation period (p₂<0.05-0.001), which indicates a positive impact of our method on these indicators.

The data given in the Table 2 demonstrates that there are disturbances of mineral composition of oral liquid in patients with periodontitis of the group II, similar to those in the group I, but being more pronounced. In particular, concentration of Fe before treatment in subgroup II A was in 1.36 times lower than the normal value (p₁<0.001).

The study suggests that there was a convincing increase of Fe content in the oral liquid, reaching the data of healthy

people in the long-term follow-up (p₂<0.001) as a result of our comprehensive therapeutic measures in these patients. In subgroup II B, the concentration of Fe before treatment was in 1.47 times lower than in healthy subjects. After treatment, it increased in 1.38, 1.40 and 1.39 times immediately, 6 and 12 months after, respectively (p₂<0.001).

The growth of Cu was in 1.26 times higher in subgroup II A compared to healthy individuals (p₁<0.001). Complex therapy in these patients induced the results close to the norm (p₁>0.05) at all periods of observation (p₂<0.001; p₂<0.005; p₂<0.01). Thus, the elevated in 1.34 times (p₁<0.001) content of Cu in the oral liquid, patients from subgroup II B decreased sharply in 1.25 times immediately after treatment, improved for another 6 months and almost did not change 12 months after (p₂<0.001; p₃>0.05).

Periodontitis was accompanied by a significant (p₁<0.001) decrease of Zn in the oral liquid of patients in both subgroups in 1.30 (II A) and 1.38 (II B) times. Complex therapy contributed to a raise of its content in all patients (p₂≤0.001), especially being pronounced 6 and 12 months after in subgroup II A, with almost complete normalization of the indicator (p₁>0.05). And in subgroup II B, the level of Zn after treatment advanced in 1.28; 1.30 and 1.27 times (p₂<0.001) and also reached a norm (p₁>0.05).

Table 2. Changes of biometals in the oral liquid of patients with periodontitis stage II, grade A and B due to the complex treatment (M±m)

Indicators	Healthy n=17	Subgroup II A				Subgroup II B			
		before treatment, n=21	after treatment n=21	6 months after, n=19	12 months after, n=16	before treatment, n=23	after treatment n=23	6 months after, n=23	12 months after, n=20
Fe, mcg/l	763.00± 21.14	560.85± 24.24 p ₁ <0.001	711.59 ±18.05 p ₁ >0.05 p ₂ <0.001	750.10± 14.89 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	729.17± 15.92 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	518.11 ±26.87 p ₁ <0.001	713.79± 17.81 p ₁ >0.05 p ₂ <0.001	724.81± 16.49 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	718.77± 15.32 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05
Cu, mcg/l	80.25± 2.65	100.79 ±4.06 p ₁ <0.01	79.52± 2.71 p ₁ >0.05 p ₂ <0.001	81.67± 2.66 p ₁ >0.05 p ₂ <0.005 p ₃ >0.05	83.01± 2.92 p ₁ >0.05 p ₂ <0.01 p ₃ >0.05	107.26 ±4.14 p ₁ <0.001	86.05± 2.75 p ₁ >0.05 p ₂ <0.001	84.42± 2.74 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	87.27± 2.72 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05
Zn, mcg/l	521.59± 14.64	402.08 ±17.11 p ₁ <0.001	492.90± 13.95 p ₁ >0.05 p ₂ <0.01	513.71± 13.36 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	513.74± 14.74 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	378.11 ±15.28 p ₁ <0.001	483.98± 11.92 p ₁ >0.05 p ₂ <0.001	489.37± 13.59 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	480.65± 13.49 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05
Mn, mcg/l	26.16± 1.38	12.96 ±1.48 p ₁ <0.001	21.81± 1.33 p ₁ >0.05 p ₂ <0.01	21.11± 1.15 p ₁ >0.05 p ₂ <0.01 p ₃ >0.05	21.30± 1.69 p ₁ >0.05 p ₂ <0.01 p ₃ >0.05	13.21 ±1.58 p ₁ <0.001	24.24± 1.35 p ₁ >0.05 p ₂ <0.001	23.14± 1.17 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05	22.32± 1.27 p ₁ >0.05 p ₂ <0.001 p ₃ >0.05

Note. See table 1.

Source: compiled by the authors of this study

According to the data gained from our study, there was the largest deviations from the norm of the trace element Mn observed in patients of the II group: in subgroup II A it was reduced in 2.01 times, and in II B – in 1.98 (p₁<0.001). The recommended therapeutic complex proved to be successful: the achieved immediately after the treatment levels of Mn were maintained in all patients of the II group (p₂<0.001), especially in the II B subgroup, moreover, over the long-term follow-up the obtained indicators were the closest to the data in healthy individuals (p₁>0.05).

Via correlation analysis we established also that there were six strong reliable (p<0.05) correlations between the indicators of biometals in the oral liquid in the patients with periodontitis before treatment. Among them, there were three direct ones: between the content of Fe and Zn (r = 0.99), Fe and Mn (r = 0.98), Zn and Mn (r = 0.98) and three inverse ones: between the level of Cu and Fe (r = -0.98); Cu and Zn (r = -0.98) and Cu and Mn (r = -0.93). There were two strong reliable correlations found after treatment, in particular: between Fe and Zn and Fe and Mn immediately (r=0.94; r=0.94) and after six months (r=0.94; r=0.89). There were three reliable correlations between the contents of Fe and Zn (r=0.89), Fe and Mn (r=0.94), Cu and Zn (r=-0.83) established after a year.

DISCUSSION

Changes in the macro- and microelement composition of biological fluids, such as blood serum and saliva may indicate pathological changes in periodontal tissue according to modern scientific data [8-9]. After all, mineral components such as Calcium, Magnesium, Iron, Selenium and Zink are responsible for normal processes in periodontal tissues, and their imbalance indicates a violation of osteosynthesis, collagen synthesis as well as alveolar bone destruction.

The role of Fe as an irreplaceable element is not the hemoglobin formation only, but also Fe being an important component of various enzyme systems that ensure tissue respiration and immune responses. The optimal level of Fe maintains alveolar bone homeostasis, cellular metabolism of anaerobic periodontopathogens, and the course of immune-inflammatory reactions of the macroorganism on the whole [10].

Thus, according to some researches, an increased level of free Fe can act as a pro-oxidant and catalyze Fenton's reaction, which leads to the reactive oxygen species (ROS) from neutrophils and macrophages [11]. This, in turn, causes the activation of matrix metalloproteinases and the release of pro-inflammatory cytokines, which participate in the destruction of periodontal tissues and resorption of alveolar bone [12].

The obtained by us results indicate the normalization of Fe in the oral liquid after the recommended treatment, which can be considered as an increase of antioxidant protection and cellular immune reactions. At the same time, some researchers note a growth of Fe in the saliva of patients with generalized aggressive periodontitis compared to individuals with healthy periodontium [8].

Thus, both increased and decreased levels of Fe are harmful to the immune response of the host's organism, susceptibility to infections, alveolar bone remodeling, therefore maintaining its optimal level is an important task in the prevention and treatment of periodontal disorders.

Analyzing the results regarding the increase of Cu in the oral liquid of patients with periodontitis compared to persons with healthy periodontium, it should be noted that this fact is consistent with the data of most researchers as Cu is a part of various enzymes and proteins that regulate Fe metabolism and lipid metabolism, as well as the synthesis of connective tissue [4, 9]. Thus, Cu is also present in the active center of lysyl oxidase, an enzyme that forms cross-links between polypeptide chains of collagen and elastin. Therefore, Cu deficiency can lead to the formation of defective collagen [13]. Furthermore, this trace element is important for the proper functioning of the immune system and for the formation of antioxidant status.

After the comprehensive therapy proposed by us, the concentration of Cu in people with periodontitis significantly decreased compared to the indicators before treatment and remained at the level of healthy people even 12 months after therapy.

The results of our study demonstrate a reduction of the Zn content in the oral fluid of patients with periodontitis compared to people with intact periodontium. This statement is consistent with the conclusions of other researchers [14]. The findings of these researchers declare that levels of redox-active metal ions, including Zn, Cu, Fe, Se, and Mn correlate strongly with SOD in saliva and to a lesser extent to SOD in serum which implies a critical role of these redox-active micronutrients in modulating oxidative stress status in periodontitis. On other hand, some scientific data report about increase Zn in saliva with periodontal disease [8]. This suggests that Zn, which is found in the saliva, plaque, and pellicle in different concentrations, limits acid production in dental plaque and inhibits the colonization of bacterial pathogens.

As a result of appointed therapy, there was a raise in the concentration of Zn in the oral fluid of patients to the level of healthy individuals. Thus, the optimal content of Zn is a necessary condition for the functioning

of immune protective mechanisms and periodontal bone tissue regeneration, which will further prevent the progression of periodontitis.

Our research signifies a decline of Mn in the oral liquid of patients with periodontitis compared to healthy individuals and are consistent with the data of other researchers [15]. At the same time, some scientists observed a notable advance of Mn in the oral fluid of patients with periodontitis compared to people with healthy periodontium [8-9]. It is well known that Mn deficiency leads to an overall decrease of antioxidant enzymes, superoxide dismutase (Mn-SOD) in particular, which protects against free radicals. An abate of the antioxidant properties of Mn-SOD increases the destruction of connective tissue and alveolar bone by reactive oxygen. The rise of Mn in the saliva after the treatment proposed by us testifies to its positive effect on the activation of antioxidant processes in periodontal tissues.

To summarize, it should be noted that the use of an agent based on the microalgae *Spirulina platensis* as a part of complex treatment contributed to the regulation of the detected disorders, especially being successful in subgroups I A, II B and II A. Treatment success was evidenced by a reduction of strong reliable correlations from six before treatment to two immediately and within 6 months, and up to three 12 months later. Such an expressive and long-lasting normalization of Fe, Cu, Zn and Mn in the oral liquid was due to the fact that the agent used is of natural origin based on blue-green microalgae is more easily absorbed by the body, as chelated (combined with amino acids) osteotropic biometals of *Spirulina platensis* are quickly incorporated into biometals included in the microelement exchange of periodontal bone tissue [16-17]. Such properties of spirulina allowed us to achieve positive outcome over the observation periods and contributed to long-term clinical stabilization. Other researches also demonstrate similar results regarding blood trace element indices in patients with chronic periodontitis [18].

CONCLUSIONS

1. There was a significant imbalance of osteotropic biometals, such as Fe, Cu, Zn and Mn found in the oral liquid of all patients with periodontitis compared to healthy individuals ($p1 < 0.005-0.001$), except for the content of Cu in patients with periodontitis stage I severity and slow rate of progression. Changes in mineral composition depended on the stage of severity and the rate of progression of the disease and were amplified in individuals with periodontitis of stage I severity and moderate rate of progression, and even

more so in patients with periodontitis stage II severity and moderate rate of progression. They demonstrated a notable decreased content of Fe, Zn and Mn and an increased amount of Cu in oral fluid.

2. The administration of the proposed complex non-surgical treatment with pharmacological agent *Spirulina platensis* and the developed paste on its

basis contributed to the steady stabilization of homeostasis of microelements, especially Fe, Zn and Mn (amount of which expressively increased) at all observation periods, both immediately and 6 and 12 months after in patients with periodontitis. The obtained data were close to the indicators of healthy people ($p > 0.05$).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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