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Research Article

Changes of micro elements, activity of metalloenzymes-antioxidants and metal-dependent enzymes in the blood serum of patients with periodontitis influenced by complex non-surgical treatment

Halyna Melnychuk[1], Veronika Kateryniuk[1], Hanna Semeniuk[1], Nataliia Shovkova[1], Roksolana Kashivska[1]

1 *Ivano-Frankivsk National Medical University, 2 Grushevskogo str., Ivano-Frankivsk, 76000, Ukraine*

Corresponding author: Hanna Semeniuk (annasemenyukd@gmail.com)

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Abstract

Data provided by WHO show that the prevalence of aggressive periodontitis is observed in almost 19% of the adult world population and is associated with the destruction of the attachment apparatus and premature tooth loss. Development of a periodontal pocket and foci of infection also negatively affect the body on the whole. Despite the great number of the researches in this field and the dramatic improvement in the treatment and prophylaxis, the problem of high prevalence of periodontal diseases remains actual. The article demonstrates the biochemical changes of Iron, Copper, Zinc, Manganese and metal-dependent oxidant-antioxidant enzymes in the blood serum of patients diagnosed with periodontitis gained after complex periodontal therapy with Spirulina platensis of blue-green microalgae. It was established that the micro- and macro-elements in the introduced agent significantly contribute to the correction of the Iron, Copper, Zinc and Manganese content, they also influence the activity of metaloproteins therefore, normalize the antioxidant defense of the body in general.

Keywords

periodontitis, serum, metalloenzymes, pro-oxidant protection, complex periodontal therapy

Introduction

Periodontitis is a poly-etiological disease characterized by a long, progressive course, bone destruction, disturbed bone remodeling and early tooth loss. The disease is accompanied by microbiological, immunological, patho-physiological and biochemical changes in the periodontium and in the body in general (Bui et al. 2019). The findings provided by authors (Mohanty et al. 2019; Chigasaki et al. 2021) suggest that a leading role in the occurrence and progression of periodontitis have the microorganisms of the red complex such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola*. Their toxins and decay products from periodontal pockets cause bacteremia and chroniosepsis. Such condition produces the tension in the detoxification system, even in somatically healthy people, which is accompanied by an increase of free radical lipids oxidation and the cell membranes damages.

Data of researchers (Dommisch et al. 2018) show that the balanced levels of trace elements such as Iron (Fe), Zinc (Zn), Selenium (Se) and Copper (Cu) are essential to arrest progression of periodontitis. Besides, some elements, such as Se, Zn and Cu are integral elements of antioxidant enzymes and prevent the tissue destruction with active oxygen. Their deficiency can worsen the course of periodontitis in those with systemic diseases such as diabetes (Gaur and Agnihotri 2017; Thomas et al. 2019).

Considering all mentioned above, it can be recommended to administer agents with large spectrum of micro- and macro-elements that provide sufficient antioxidant, antibacterial and osteotropic effect in order to arrest periodontal inflammation and bone destruction as well as to normalize the micro-, macro- elements indexes and to improve metalloenzyme's metabolism and lipid's peroxidation. The search for medications that regulate disordered homeostatic parameters remains certain.

The article presents the changes of trace elements Fe, Cu, Zn, Mn and the activity of metalloenzymes-antioxidants and metal-dependent enzymes (ceruloplasmin, catalase, lactate dehydrogenase, iron saturation of transferrin, alkaline and acid phosphatases) in the blood serum of patients with periodontitis obtained after administration of complex non-surgical treatment.

Materials and methods

A total of 169 non-smokers aged 25–44 years were enrolled in this study. They were divided into three groups: 41 healthy people with intact periodontium, 65 patients diagnosed with periodontitis stage II grade A (slow progression) and 63 individuals with periodontitis stage II grade B (moderate progression). Patients diagnosed with periodontitis were examined before treatment, after, after half a year and a year.

The content of biometals such as Fe, Cu, Zn and Mn in whole blood was investigated on an atomic adsorption spectrophotometer S-115 PC by the standard method (Pogorelov et al. 2010). Blood was collected from the elbow vein before breakfast in the morning. Part of the blood was separated, centrifuged and serum was aggregated, in which the activity of antioxidant enzymes was determined: catalase was established according to AN Bakh and SV Zubkova method, iron saturation of Trasferrin (TF) and activity of the Ceruloplasmin (CP) according to GO Babenko method (Babenko 1968), activity of metal-dependent Lactate dehydrogenase enzymes (LDH) – by using standard sets of biotests and technique of the company Lachema, (Czech Republic); Alkaline phosphatase (ALP) – with Bio-Ld-TEST kits (Lachema, Czech Republic) and Acid phosphatase (AP) – with Simko Ltd (Lviv) kits.

All patients with periodontitis were treated comprehensively, using traditional initial periodontal therapy (required in each clinical situation) and the treatment with a paste developed by us. The paste consisted of equal parts of "Spirulina" which is based on the blue-green microalgae *Spirulina platensis* and silica enterosorbent "Sylard-P" mixed ex tempore with a 0.05% solution of Chlorhexidine bigluconate to a gel like consistency. The mixture was applied to the gums and directly inserted into the periodontal pockets for 20–30 minutes over the course of 6–8 days, after 1–2 days. Silard-P is a medical sorbent approved for clinical use by the Pharmaceutical Committee of Ukraine (protocol No. 1 dated January 23, 1993). It is a light white powder, the bulk weight of which is 40–60 g/l. The size of the particles that make up the powder ranges from 5 to 20 nm. The specific surface of the sorbent is significant and reaches 300 m^2 per 1 g of substance. Due to its high sorption capacity, Silard-P is able effectively remove harmful products of endo- and exogenous origin from the body. Its specific surface is 300 m^2 per 1 g of substance (Voronin et al. 2017). This fact determines its high adsorption capacity. Thus, Silard-P binds up to 300–800 mg/g of protein substances and more than 109 microorganisms per gram, and this at a fairly high rate of adsorption, which reaches 80% of its adsorption capacity in 1 minute of the incubation. At the same time, desorption from the surface of Silard-P is quite slow and, depending on the structure of the immobilized agents, takes up to 2–3 days (Voronin et al. 2016).

The recommended dose 1.0 g of "Spirulina" was prescribed to be taken twice daily 20 minutes before meal for 4 weeks. "Spirulina" is 100% blue-green microalgae Spirulina platensis. Spirulina contains the most vital vitamins in optimal ratios (including antioxidants - A, B_1, B_2, B_3, B_6, PP, biotin, folic acid, inositol, pantothenate, C and E, 28 amino acids (including 8 essential ones: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine), more than 11 unsaturated acids, as well as numerous macro- and microelements, namely: Sodium, Potassium, Magnesium, Chlorine, Phosphorus, Iron, Zinc, Copper, Iodine, Selenium and other useful substances: isoprenoids, enzymes, indole, phenolic compounds.

Supportive therapy was carried out six month after and consisted of the initial periodontal therapy with administration of a locally delivered complex recommended by us. A year later, a course of "Spirulina" was prescribed again. Patients with periodontitis II-degree stage B were also given local sustenance therapy.

This study was conducted in agreement with the Declaration of Helsinki-Ethical principle for medical research involving human subjects.

Statistical processing of the results was carried out by a personal computer based on an application program for Microsoft Excel spreadsheets and "Statistica 6.0" package, using the methods of descriptive statistics and correlation analysis. The results were considered probable when the confidence coefficient was less than or equal to 0.05.

Research results and their discussion

Examination of all patients with periodontitis demonstrates that the content of Fe in their blood was decreased in 1.12 times; $p_1<0.001$ (Table 1). However, the amount of this metal has dramatically increased in 1.08 times immediately and in 1.09 times in 6 and 12 months ($p_2<0.001$) after appointed therapy.

The level of Cu, on the contrary, was increased in 1.12 times ($p_1<0.001$). Our measures contributed to its significant reduction in 1.01 times ($p_2<0.001$), reaching the indicators in healthy people and being maintained during the year ($p_2<0.001$; $p_3>0.05$; $p_4>0.05$).

The concentration of Zn in the blood of patients with periodontitis was reduced in 1.26 times ($p_1<0.001$). As a result of complex therapy, it probably increased in 1.22 times ($p_2<0.001$) immediately, remained at this level for six months and decreased slightly after a year, but remained reliably different from the initial data (in 1.19 times; $p_2<0.001$). Although the gained Zn indicators approached those of healthy people ($p_1>0.05$), their complete normalization did not occur.

At the same time, the amount of Mn, which was in 1.36 times lower than in healthy people ($p_1<0.001$), increased significantly in 1.35 times ($p_2<0.001$) due to the complex treatment administered, then slightly fluctuating, remained at this level for a year , almost reaching the norm.

By analyzing the data in Table 2, it can be assumed that in case of periodontitis degree II stage A the level of Fe increased in 1.09, 1.11, and in 1.10 times influenced by appointed treatment, and in case of periodontitis degree II stage B indicators increased in 1.10, 1.12 and in 1.11 times immediately and 6, 12 months after, respectively ($p_1<0.001$). The indicators of Fe content obtained immediately after therapy continued to rise after 6 months, and changed a little after 12 months ($p_2>0.05$; $p_3>0.05$).

The study suggests that there was a significant decrease in 1.17 times ($p_1<0.001$) of Cu in the blood of patients with periodontitis degree II stage A as a result of conducted therapy. Moreover, the indicators achieved immediately and 6 months after, became even lower than in healthy people and corresponded to their data in 12 months. In case of patients with periodontitis degree II stage B there was a decrease of Cu immediately in 1.16, 1.15 times and in 1.15 times ($p_1<0.001$) 6 and 12 months after. The indicators have become close to the norm (see Table 1).

Further study show that the complex treatment proved to be successful to regulate Zn in the blood of patients with periodontitis degree II stage A, which level increased sharply immediately and 6 months later (in 1.24 times; $p_1<0.001$) and slightly decreased 12 months then. The regulation of Zn content in case of patients with periodontitis degree II stage B was also significant, but the figure achieved immediately declined slightly in the long terms, although the difference with the data before therapy (in 1.22, 1.22 and in 1.18 times) was convincing at all stages of the investigation (p_1 <0.001).

Table 1. Dynamics of trace elements in the blood of all examined patients with periodontitis II degree stage A and stage B obtained after complex treatment (M±m).

Indicators	Healthy	Patients with periodontitis			
		Before treatment	After treatment	In 6 month	In 12 month
Fe, mg/l	n=27 520,83±10,32	n=75 464,51±4,42 $p_1<0{,}001$	n=74 501,24±3,94 $p_1>0{,}05$ $p_2<0{,}001$	n=61 508,49±4,51 $p_1>0{,}05$ $p_2<0{,}001$ $p_3>0{,}05$	n=53 504,97±4,82 $p_1>0{,}05$ $p_2<0{,}001$ $p_3>0{,}05$ $p_4>0{,}05$
Cu, mg/l	n=28 1,37±0,02	n=74 1,53±0,02 $p_1<0{,}001$	n=74 1,36±0,02 $p_1>0{,}05$ $p_2<0{,}001$	n=61 1,36±0,02 $p_1>0{,}05$ $p_2<0{,}001$ $p_3>0{,}05$	n=53 1,38±0,02 $p_1>0{,}05$ $p_2<0{,}001$ $p_3>0{,}05$ $p_4>0{,}05$
Zn, mg/l	n=32	n=76 5,57±0,08 $p_1<0{,}001$	n=74 6,77±0,09 $p_1>0{,}05$ $p_2<0{,}001$	n=61 6,74±0,09 $p_1>0{,}05$ $p_2<0{,}001$ $p_3>0{,}05$	n=53 6,64±0,09 $p_1>0{,}05$ $p_2<0{,}001$ $p_3>0{,}05$ $p_4>0{,}05$
Mn, mcg/l	n=41 87,32±1,72	n=76 64,09±1,11 $p_1<0{,}001$	n=74 86,44±1,05 $p_1>0{,}05$ $p_2<0{,}001$	n=61 85,59±1,21 $p_1>0{,}05$ $p_2<0{,}001$ $p_3>0{,}05$	n=53 86,18±1,11 $p_1>0{,}05$ $p_2<0{,}001$ $p_3>0{,}05$ $p_4>0{,}05$

Note. Table 1 and table 3 show the probability of indicators: p_1 - to the value in healthy people; p_2 – to the value before treatment; p_3 – to the value after treatment; p_4 - to the value after 6 months.

Table 2. Indicators of biometals in the blood of patients with periodontitis obtained immediately after complex treatment (M±m).

Indicators	Patients with periodontitis degree II stage A				Patients with periodontitis degree II stage B			
	Beforetreatment	After treatment	In 6 months	In 12 months	Beforetreatment	After treatment	In 6 months	In 12 months
Fe, mg/l	n=34 462,68±4,55	n=32 503,83±5,56 p_1<0,001	n=315 12,01±5,93 p_1<0,001 p_2>0,05	n=30 507,82±6,52 p_1<0,001 p_2>0,05 p_3>0,05	n=41 452,32±4,34	n=40 499,16±5,56 p_1<0,001	n=30 504,72±6,88 p_1<0,001 p_2>0,05	n=27 502,22±7,15 p_1<0,001 p_2>0,05 p_3>0,05
Cu, mg/l	n=32 1,57±0,03	n=32 1,34±0,03 p_1<0,001	n=31 1,34±0,03 p_1<0,001 p_2>0,05	n=30 1,37±0,02 p_1<0,001 p_2>0,05 p_3>0,05	n=42 1,60±0,02	n=42 1,38±0,03 p_1<0,001	n=30 1,39±0,02 p_1<0,001 p_2>0,05	n=27 1,39±0,03 p_1<0,001 p_2>0,05 p_3>0,05
Zn, mg/l	n=33 5,60±0,08	n=32 6,92±0,13 p_1<0,001	n=31 6,93±0,11 p_1<0,001 p_2>0,05	n=30 6,86±0,11 p_1<0,001 p_2>0,05 p_3>0,05	n=43 5,45±0,07	n=42 6,65±0,13 p_1<0,001	n=30 6,55±0,13 p_1<0,001 p_2>0,05	n=27 6,43±0,13 p_1<0,001 p_2>0,05 p_3>0,05
Mn, mcg/l	n=34 65,05±1,05	n=32 87,59±1,60 p_1<0,001	n=31 85,17±1,75 p_1<0,001 p_2>0,05	n=30 86,10±1,69 p_1<0,001 p_2>0,05 p_3>0,05	n=42 61,22±1,12	n=42 85,56±1,40 p_1<0,001	n=30 86,02±1,68 p_1<0,001 p_2>0,05	n=27 86,27±1,47 p_1<0,001 p_2>0,05 p_3>0,05

Note. Here and in Table 4 is shown the probability of the difference in indicators: p_1 – to the value before treatment; p_2– to the value after treatment; p_3 - to the value 6 months after treatment.

There are a slightly different regularities regarding Mn in the blood of patients with periodontitis degree II stage B its level, which was reduced before treatment, advanced throughout the year, while in case of periodontitis degree II stage A, the highest Mn content was recorded immediately after the end of therapy.

However, the treatment proved to be successful in all patients, and the obtained indicators were almost the same as in healthy individuals.

Furthermore, we also found that the activity of metallo-enzymes and metallo-dependent enzymes in the blood serum experienced changes in all patients with periodontitis (Table 3). Thus, catalase activity was reduced in 1.14 times (p_1<0.001). As a result of therapy, it advanced sharply in 1.13 times (p_2<0.001), even more 6 months afterwards (in 1.14 times; p_2<0.001), reaching the level of healthy people, and slightly lessening next, it remained in 1.11 times (p_2<0.001) higher than the initial data after a year.

The index of iron saturation of TF was also significantly lower in 1.15 times (p_1<0.001) in patients with periodontitis than that of healthy people, but the implemented measures proved to have a positive effect immediately after therapy, as TF iron saturation increased in 1.13 times (p_2<0.001) and slightly declined 6 months afterwards, but remained significantly high compared to the initial data (p_2<0.001). A slight reduction of this indicator continued a year thereafter, but the probable difference with the data before treatment remained and amounted up to 1.07 times (p_2<0,05), however, the difference from the statistic obtained immediately became significant (p_3=0,001).

The level of CP activity in patients with periodontitis was elevated in 1.18 times (p_1<0.001). Due to the treatment appointed, it became reduced in 1.17 and 1.16 times immediately and after six months (p_2<0.001) and then reaching the norm. After a year, the activity of the CP climbed, but the difference in 1.11 times remained indicative (p_2<0.05) comparing with the data before therapy, and in comparison with the same index of healthy people -insignificant (p_1>0.05). The activity of the metal-dependent enzyme LDH differed in 1.47 times (p_1<0.001) from healthy people. Treatment successfully affected this indicator: it immediately decreased in 1.21 times (p_2<0.001), and in 6 months its reduction continued (in 1.31 times; p_2<0.001). Later than 12 months, LDH advanced and the contrast with data in healthy people became indicative (p_1<0.05), but comparing with the initial indicator remained significant (in 1.16 times; p_2<0.001). At the same time, the level of LDH obtained after one year was more notable from that observed after six months (in 1.13 times; p_4=0.01).

There are also changes in ALP activity evidenced due to the appointed therapy as it was reduced before treatment in 1.08 times (p_1 <0.001), and after therapy it increased immediately and corresponded to the norm and amounted to (1.38±0.02) mkkat/l. The obtained result was maintained for half a year and changed a little in a year (p_1>0.05; p_2 <0.001; p_3>0.05; p_4>0.05).

Periodontitis was presented by a raised activity of AP in the blood serum in 1.28 times (p_1<0.001). With our comprehensive measures, it became possible to reduce it in 1.16, 1.13 and 1.09 times, immediately, after 6 and 12 months (p_2<0.001). However, the data of healthy people could not be reached in any observation period and the difference remained probable (p_1<0.005; p_1<0.001). The difference between AP activity achieved directly and 6 months later treatment was also convincing (p3<0.05).

Table 3. Dynamics of the activity of metalloenzymes and metal-dependent enzymes in the blood serum of patients with periodontitis II degree stage A and stage B under the influence of complex treatment (M±m).

Indicators	Healthy	Patients with periodontitis			
		Before treatment	After treatment	In 6months	In 12 months
Catalase, c.u.	n=32 14,77±0,48	n=128 12,90±0,22 p_1<0,001	n=123 14,55±0,16 p_1>0,05 p_2<0,001	n=115 14,72±0,20 p_1>0,05 p_2<0,001 p_3>0,05	n=113 14,32±0,18 p_1>0,05 p_2<0,001 p_3>0,05 p_4>0,05
TF c.u.	n=34 0,196±0,004	n=111 0,171±0,001 p_1<0,001	n=110 0,194±0,002 p_1>0,05 p_2<0,001	n=104 0,189±0,002 p_1>0,05 p_2<0,001 p_3>0,05	n=99 0,183±0,002 p_1>0,05 p_2<0,05 p_3=0,001 p_4>0,05
CP c.u.	n=36 31,28±0,92	n=110 36,85±0,95 p_1<0,001	n=108 31,45±0,65 p_1>0,05 p_2<0,001	n=101 31,64±0,70 p_1>0,05 p_2<0,001 p_3>0,05	n=97 33,12±0,77 p_1>0,05 p_2<0,05 p_3>0,05 p_4>0,05
LDH mkkat/l	n=27 1,24±0,13	n=102 1,82±0,04 p_1<0,001	n=98 1,50±0,04 p_1>0,05 p_2<0,001	n=93 1,39±0,05 p_1>0,05 p_2<0,001 p_3>0,05	n=93 1,57±0,04 p_1<0,05 p_2<0,001 p_3>0,05 p_4=0,01
ALP mkkat/l	n=27 1,38±0,04	n= 125 1,28±0,01 p_1<0,001	n=122 1,38±0,02 p_1>0,05 p_2<0,001	n=120 1,37±0,01 p_1>0,05 p_2<0,001 p_3>0,05	n=116 1,35±0,02 p_1>0,05 p_2<0,001 p_3>0,05 p_4>0,05
AP nmol/c/l	n=32 149,19±3,93	n=123 191,55±2,94 p_1<0,001	n=122 164,57±3,49 p_1<0,005 p_2<0,001	n=110 168,81±3,43 p_1<0,001 p_2<0,001 p_3>0,05	n=108 174,96±3,36 p_1<0,001 p_2<0,001 p_3<0,05 p_4>0,05

Note. Here and in the table. 3 the probability of indicators are shown: p_1- to the value in healthy people; p_2– to the value before treatment; p_3– to the value after treatment; p_4 - to the value after 6 months.

Having analyzed the changes of the catalase metalloenzyme activity influenced by introduced treatment in patients with periodontitis (Table 4), it can be assumed that in patients with periodontitis stage A and stage B, the indicators differed only slightly from each other. As the figures suggest the therapy contributed to the almost complete normalization of the enzyme activity at all observation, especially in patients with periodontitis stage B 6 months afterwards.

The index of Iron saturation of TF was regulated somewhat less than Catalase activity. However, its growth was indicative at all periods of observation: in 1.14, 1.10 and 1.07 times in patients with periodontitis stage A and in 1.12, 1.10 and 1.06 times in in patients with periodontitis stage B (p_1<0.001; p_1< 0.005). Transferrin demonstrated the best result of iron saturation immediately after the end of therapy in patients with periodontitis stage A, which was even higher than in healthy people (see Table 3).

CP experienced also notable changes as its activity has lowered immediately and 6 months after in 1.17 times (p_1<0.001) in patients with periodontitis stage A. The obtained indicators became smaller than in healthy people and indicate a complete normalization of its activity. After a year, it increased slightly, but remained significantly different from the initial data (p_1<0.001). In in patients with periodontitis stage B, the indicators also reduced in 1.17, 1.16 and 1.20 times immediately, 6 and 12 months after (p_1<0.001). Steady regulation of this indicator has been observed for 6 months.

There was a reduction of the LDH observed in the blood serum of patients with periodontitis stage A and stage B (p_1<0.001). It is interesting to note that it was at its lowest in all patients after 6 months, although it was not possible to completely stabilize the achieved data. A year behind its activity increased again, and the difference with the data obtained after 6 months became significant in both groups (p_3<0.001).

Table 4. Changes of metalloenzymes and metallo-dependent enzymes activity in the blood serum of patients with after complex treatment (M±m).

Indicators	Patients with periodontitis degree II stage A				Patients with periodontitis degree II stage B			
	Before treatment	After treatment	In 6 months	In 12 months	Before treatment	After treatment	In 6 months	In 12 months
Catalase, c.u.	n=65 12,86±0,32	n=63 14,60±0,21 p_1<0,001	n=58 14,68±0,26 p_1<0,001 p_2>0,05	n=57 14,36±0,22 p_1<0,001 p_2>0,05 p_3>0,05	n=63 12,94±0,30	n=60 14,50±0,23 p_1<0,001	n=57 14,75±0,30 p_1<0,001 p_2>0,05	n=56 14,29±0,28 p_1<0,001 p_2>0,05 p_3>0,05
TF c.u.	n=56 0,173±0,002	n=55 0,197±0,004 p_1<0,001	n=53 0,191±0,003 p_1<0,001 p_2>0,05	n=50 0,185±0,003 p_1<0,005 p_2>0,05 p_3>0,05	n=55 0,170±0,002	n=55 0,191±0,003 p_1<0,001	n=51 0,187±0,003 p_1<0,001 p_2>0,05	n=49 0,181±0,003 p_1<0,001 p_2<0,05 p_3>0,05
CP c.u.	n=55 36,06±1,02	n=54 30,83±1,02 p_1<0,001	n=51 30,89±1,01 p_1<0,001 p_2>0,05	n=47 32,64±1,03 p_1<0,001 p_2>0,05 p_3>0,05	n=55 37,64±0,95	n=54 32,06±0,82 p_1<0,001	n=50 32,40±0,95 p_1<0,005 p_2>0,05	n=50 33,58±1,13 p_1<0,001 p_2>0,05 p_3>0,05
LDH mkkat/l	n=50 1,74±0,06	n=49 1,49±0,05 p_1<0,001	n=47 1,37±0,06 p_1<0,001 p_2<0,05	n=47 1,53±0,06 p_1<0,001 p_2>0,05 p_3<0,001	n=52 1,91±0,07	n=49 1,52±0,06 p_1<0,001	n=46 1,41±0,07 p_1<0,001 p_2<0,05	n=46 1,60±0,06 p_1<0,001 p_2<0,05 p_3<0,001
ALP mkkat/l	n=63 1,28±0,01	n=61 1,38±0,03 p_1<0,001	n=61 1,37±0,02 p_1<0,001 p_2>0,05	n=58 1,35±0,02 p_1<0,001 p_2>0,05 p_3>0,05	n=62 1,27±0,02	n=61 1,38±0,02 p_1<0,001	n=59 1,36±0,02 p_1<0,001 p_2>0,05	n=58 1,35±0,02 p_1<0,001 p_2>0,05 p_3>0,05
AP nmol/c/l	n=62 178,42±3,34	n=61 153,79±4,49 p_1<0,001	n=55 161,49±4,38 p_1<0,001 p_2<0,005	n=55 164,93±4,26 p_1<0,005 p_2<0,005 p_3>0,05	n=61 204,90±4,25	n=61 175,34±5,01 p_1<0,001	n=55 176,13±5,12 p_1<0,001 p_2>0,05	n=53 185,38±4,86 p_1<0,001 p_2<0,05 p_3<0,05

The positive effect of complex treatment was also manifested by a growth and complete normalization of ALP activity in patients with periodontitis stage A and stage B directly after appointed therapy (p_1<0.001) and with a minimal decrease in the long-term follow-up with indicators close to normal.

At the same time, the increased AP activity of patients with periodontitis was also successfully regulated: it decreased in 1.16, 1.10 and 1.08 times in patients with periodontitis stage A (p_1<0.001; p_1<0.005) and in 1.17, 1.16 times in patients with periodontitis stage B (p_1<0.001) not reaching the index of the healthy people.

There were reliable strong correlations between all indicators of biometals and their depended enzymes found in patients with periodontitis. Thus, the relationship between the amount of Fe and Catalase activity, TF and ALP was direct (r>0.93–0.97; p<0.001), while with CP, LDH and AP – inverse (r>0.78- 0.95; p<0.05–0.001). Cu index had close direct relationships with activity of CP and AP (r≥0.86–0.93; p<0.01–0.001) and indirect with Catalase, TF, LDH and ALP (r>-0.83 -0.95; p<0.005–0.001). The Zn content was positively correlated with the Catalase activity, TF and ALP (r>0.88–0.95; p<0.005–0.001), and negatively - with CP, LDH and AP (r>-0.74–0.90; p<0.05–0.005). Close direct correlations were revealed between Mn level and Catalase activity, TF and ALP (r>0.93–0.98; p<0.001), while CP, LDH and AP were inverse (r>-0.83–0, 98; p<0.05–0.001). In total, 24 strong reliable correlations were established.

The number of close correlations sharply decreased to four immediately after treatment: between Fe and ALP (r>0.94; p<0.005) and CP (r>-0.83; p<0.05), as well as between Zn and TF (r>-0.83; p<0.05) and CP (r>-0.99; p<0.001). There were six of them in 6 months: reliable correlations between Fe and CP and Zn and TF and CP remained and the correlation between Fe and ALP disappeared, whilst the new ones between Fe and TF and LDH, as well as Mn and ALP were added (p<0.05–0.005). There were nine of them a year after: the connections between Zn, LDH and ALP, as well as Cu and ALP (p<0.05) added to those already mentioned.

To sum up, the number of correlations has been reduced in six times soon after complex therapy, in four times - after 6 months, and in 2.7 times - after a year, testifying success of the therapy used, which contributed to a fairly stable regulation of the microelement and metalloenzyme spectrum of the blood, which ensured the stabilization of periodontitis.

Discussion

It is important to study the content of micro- and macroelements in order to understand the pathogenetic links of periodontitis. The investigated trace elements are considerable activators and cofactors of many metalloenzymes that directly and indirectly affect the free radical oxidation and the body's antioxidant defense.

Iron is an irreplaceable trace element that plays a crucial role in the metabolic processes of any living organism. Although its main function is oxygen transport, its deficiency can inhibit protective enzymes activity, such as myeloperoxidase as necessary factor for phagocytosis performed by macrophages (Arnhold 2020). Optimal Fe levels are vital for the normal functioning of periodontal tissues, and a shift in either direction can be harmful. The obtained results indicate an increase of Iron and iron-containing enzymes activity due to the treatment administered. The latter also can be considered as a change of Iron qualities from pro-oxidant to antioxidant as well as strengthening of antioxidant protection in general.

According to scientific research (Dommisch et al. 2018), one of the key features of Cu is that it can exist in reduced (Cu^{+}) and oxidized (Cu^{2+}) states and thus can participate in a wide range of protein-driven biochemical reactions. Copper-containing metalloproteins, such as Cu^{2+}/Zn^{2+} superoxide dismutase, play a considerable role in maintaining of cellular redox balance and, in turn, are vital for immune function. The results of this study indicate an increased amount of Copper in the blood serum of patients with periodontitis compared to healthy people. The findings provided by authors (Thomas et al. 2019) have also shown elevated Cu levels in people with periodontitis. After the therapy the content of Cu and the activity of the associated metalloproteinase CP has decreased in biological fluids, which indicated its lessening in free radical reactions, as well as the activity of the antioxidant system.

An optimal level of Zn is necessary for the growth and development of periodontal tissues. According to data of researches (Lee 2018) the deficiency of this trace element leads to a decrease in the protection of sulfhydryl groups and an increase in the production of reactive oxygen species, its excessive levels can act as a pro-oxidant, causing a decrease in Cu-Zn superoxide dismutase in erythrocytes. The conducted biochemical studies indicate a fall of Zn in patients with periodontitis compared to individuals with intact periodontium, which is consistent with the data of other researchers (Aziz et al 2021). After the introduced non-surgical treatment, there was a growth of Zn in the blood serum compared to the initial level.

Manganese is also important in metabolism and antioxidant protection. Its deficiency affects synthesis of hyaluronic acid and chondroitin sulfate, which are essential in the formation of connective tissue, cartilage and bone (Jerosch 2011). The increase of manganese after the proposed treatment also indirectly testifies its positive effect on antioxidant processes as the rise of Catalase in blood serum in particular. The given data of biochemical studies indicate a decrease of alkaline phosphatase activity as a marker of bone formation and an increase of acid phosphatase as a marker of bone resorption in patients diagnosed with chronic periodontitis that is clearly correlated with the severity of the disease. There was a growth of ALP and a decline of acid phosphatase observed in 6 and 12 months, which indicates the stimulated osteogenesis due to treatment appointed.

The positive effect was also manifested by a significant lessening of LDH in the blood serum of patients with periodontitis. LDH is an intracellular enzyme, so an increased extracellular concentration indicates a cell death. It is observed when the attachment epithelium is destroyed; the enzyme is released into the interstitium and gingival fluid. The growth of LDH activity, which correlates with the severity of periodontitis, is confirmed by other authors (Ali et al 2018; Krajnov et al. 2018).

Conclusion

The study regarding the content of biometals show a probably decrease of Fe, Zn, Mn and an increase of Cu ($p_1<0.001$) in the blood of patients with generalized periodontitis. The results of the study suggest that the effect of complex treatment administered contributed to the successful regulation of microelement homeostasis ($p_2<0.001$): the amount of Cu and Mn was normalized (at all terms, for both variants over the course of the disease) and the content of Fe and Zn was also regulated (especially 6 months after in the case of a chronic course). Periodontitis was also accompanied by an imbalanced activity of metalloenzymes and metallodependent enzymes, in particular, a decrease of catalase, ALP and iron saturation of TF and an increase of CP, LDH and AP. The implemented therapeutic measures resulted in almost complete normalization of catalase and ALP activities during the investigation. The index of iron saturation of TF and CP activity was normalized up to six months as well. At the same time, the level of CP and AP decreased sharply ($p_2<0.001$), but it was not possible to achieve data of healthy individuals. The best results were almost often observed immediately and 6 months afterwards in case of periodontitis degree II stage A. However, catalase activity was at its highest 6 months later in case of periodontitis degree II stage B and ALP – in both variants of the course.

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