REGULATION OF ANTIOXIDANT ENZYMES IN PATIENTS AFTER PERIODONTAL TREATMENT WITH NATURAL AGENTS

Halyna M. Melnychuk, Hanna D. Semeniuk, Roxolana S. Kashivska, Natalia I. Shovkova, Nadiia S. Melnyk
IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT
The aim: The study of the possibilities of oxidase-antioxidant system indicators regulation at patients with periodontitis under the influence of complex treatment.

Materials and methods: 36 healthy and 125 patients with chronic and exacerbated periodontitis of primary (22 and 21), I (21) and II (20) degrees were examined. Indicators of lipid peroxidation and antioxidant protection (levels of diene conjugates and malonic dialdehyde, catalase activity and transferrin iron saturation, ceruloplasmin activity) in the blood serum were studied before, 6 and 12 months after the appointed treatment. Initial periodontal therapy and a paste developed by us (spirulina microalgae powders and silica enterosorbent taken in equal amounts and 0.05% chlorhexidine bigluconate) for applications and instillations were exogenously used in the complex treatment. Spirulina tablets were prescribed per os as well.

Results: All patients exhibit elevated levels of diene conjugates and malonic dialdehyde, decreased catalase activity and transferrin iron saturation as well as an increased ceruloplasmin activity, especially pronounced at stages I and II (p ≤ 0.01-0.001). Treatment contributed to long-term and reliable (p ≤ 0.05 - 0.001) regulation of the studied parameters: reduction of diene conjugates and malonic dialdehyde, ceruloplasmin activity and increased catalase activity and transferrin iron saturation. All indicators differed slightly from the norm during the year (p > 0.05), and complete normalization of most of them lasted six months. At the same time clinical stabilization of periodontitis was reached.

Conclusions: Indicators of the oxidase-antioxidant system in patients with periodontitis are significantly altered and indicate their participation in the pathogenesis of the disease. Complex treatment was able to almost completely normalize them within six months, but a year later the difference between the obtained indicators with data in healthy people was insignificant (except for ceruloplasmin). Clinical stabilization was achieved in all patients.

KEY WORDS: periodontitis, lipid peroxidation, antioxidant protection, blood serum, complex treatment

INTRODUCTION
Despite numerous studies, the pathogenesis of chronic periodontitis is not fully understood and treatment is not always effective. This necessitates the search for and development of new methods and ways of its treatment [1-3]. It is known that the development of periodontitis is associated with imbalance of pro- and antioxidant (AO) system, which causes the accumulation of reactive oxygen species and toxic metabolites, in particular, diene and triene conjugates (DC) and malonic dialdehyde (MDA) [4-8]. Due to this reduces the level of AO protection: changes the activity of catalase, transferrin (TF) and ceruloplasmin (CP), etc. [5-11]. Therefore, in the complex therapy of periodontitis it is advisable to use AO and other bioregulators [12, 13], especially of natural origin with multifaceted action.

THE AIM
The study of the possibilities of oxidase-antioxidant system indicators regulation at patients with periodontitis under the influence of complex treatment.

MATERIALS AND METHODS
161 patients aged 19 - 45 years, somatically healthy, were examined, among whom 36 people had intact periodontum and 125 were diagnosed with periodontitis. 43 patients had periodontitis of initial degree (22 - with a chronic course, 21 - with an acute course), 42 - I degree (21 persons of each course) and 40 - II degree (20 persons of each course). All patients venous blood were taken in the morning hours on an empty stomach. It was settled, centrifuged and serum was collected. The level of DC was studied by a simplified spectrometric method (Havrilov VB and coauthors) [14] and MDA – by test with 2-thiobarbioturic acid [15]. In blood serum was determined: catalase activity - according to the method Bakha AN and Zubkova SV [16], iron saturation of TF and CP activity – according to the method of Babenko GO [16]. Patients were examined before, after, six months and a year after therapy.

For complex treatment we used the method developed by us, which included exogenous and endogenous use of biologically active supplement based on blue-green microalgae Spirulina platensis. Initial periodontal therapy was performed topically corresponded to the extent necessary for each case. After that, applied for 20-30 minutes on the gums and installed in the periodontal pockets of the paste, which consisted of equal parts of spirulina powders and silica enterosorbent mixed with 0.05% solution of chlorhexidine bigluconate to a gel-like consistency. Course - 6-8 procedures through 1-2 days. It was prescribed a tablets
of spirulina 2.0-4.0 g twice a day per os, course - 4 weeks. After 6 months, we carried out individual supportive therapy and topical treatment. After 12 months, the course of general therapy was repeated, and if necessary – supportive topical treatment.

We used a personal computer and a Microsoft Excel application to process the data. The «STATISTICA 6.0» package was administrated, using descriptive statistics methods; the method of differences was administrated, using Student's t-test and correlation analysis.

The clinical study was conducted in accordance with the legislation of Ukraine and the principles of the Helsinki Declaration of Human Rights, without the participation of pharmaceutical companies.

RESULTS
According to our research (table 1), lipid peroxidation (LP) rates increase in the serum of patients with periodontitis. In particular, the level of DC in the case of chronic periodontitis initial degree was increased in 1.31 times and in exacerbated - in 1.29 (p<0.05). Under the influence of complex treatment, this figure decreased in 1.30, 1.29, 1.28 and 1.30, 1.26, 1.25 times at once, after 6 and 12 months, respectively (p<0.05) in both subgroups. The difference with the content of DC in healthy individuals in all periods of observation was not significant (p>0.05), however, in the case of an acute course after 12 months, this figure became significantly higher than immediately after treatment (p<0.05).

The amount of MDA in the serum of all patients with periodontitis of initial degree was slightly increased (p>0.05). Treatment contributed to a sharp decrease of its concentration at chronic course in 1.12 times (p<0.005), in a case of exacerbated – in 1.18 (p<0.001). At the same time, lower values were achieved than in healthy people. The difference with the data before treatment remained significant after six months and a year in all patients (p≤0.05 - 0.001), and the parameters corresponded to those in healthy people (table 1).

Analysis of the state of the prooxidant system in patients with periodontitis of the I degree showed that the concentration of DC in them was increased at the chronic course in 1.45 times (p<0.005), and in a case of exacerbated – in 1.38 (p<0.01). Complex therapy in these patients was successful at all times and the decrease in the level of DC at the chronic course was 1.43, 1.40 and 1.40 papa (p<0.01), but in a case of exacerbated – 1.30, 1.32 and 1.29 (p<0.05).

A similar regularity was observed for the content of MDA in the serum of patients with periodontitis of the first degree of the II degree. Its level in a case of chronic course was somewhat better regulated by treatment in all periods (p<0.01; p=0.001; p<0.01), than in a case of exacerbation (p<0.001; p=0.01; p<0.05). However, the data obtained were close and almost corresponded to the norm of six months for both variants of the disease.

According to the number of DC in the serum, the difference with healthy and in the case of chronic periodontitis of the second degree was 1.48 times (p<0.01). There was a decrease of it in 1.35 times as a result of complex therapy immediately (p<0.05) and even more – in 1.38 times (p<0.05) – after half of year. The difference from the original data (in 1.33 times; p<0.05) remained significant after 12 months. The level of DC in the case of exacerbated periodontitis was increased in 1.38 times (p=0.001) prior to treatment. The greatest decrease in this parameter was observed immediately after therapy (in 1.28 times; p<0.01). Subsequently, the content of DC remained significantly lower than the original data (p<0.05).

The amount of MDA in the serum was the highest among all examined patients with periodontitis of chronic course of the II degree and the difference with healthy people was in 1.19 times (p=0.001). However, treatment was able to normalize this figure immediately (p2 = 0.005) and its growth after 6 and 12 months was negligible. By the way, the difference with the data before therapy remained significant (p<0.01; p<0.001). No less successful was the treatment in the case of an exacerbated course, and patterns of reducing the level of MDA - similar (p<0.05). It is interesting to note the fact that the results achieved after treatment in these patients were close to those in periodontitis of the first degree and differed little from the norm (p>0.05).

The increased content of products of LP in the serum of patients with periodontitis was accompanied by disorders in the AO system (table 2). In particular, catalase activity in the case of chronic periodontitis initial degree decreased slightly but exacerbated - significantly (in 1.11 times; p<0.05). There was a sharp increase of it at once in 1.10 and 1.12 times (p<0.05; p<0.005) under the influence of complex treatment at chronic and exacerbated course respectively. There was some decrease in catalase activity subsequently, however, the data obtained were normal after 6 months at chronic periodontitis and were close to it in the case of exacerbated (p>0.05).

The rate of TF saturation by iron at the initial stage of periodontitis before treatment was also significantly lower than in healthy persons in both variants of the disease – in 1.08 and 1.10 times (p<0.01; p<0.001). There was an increase of it in all patients as a result of complex treatment and the patterns of these changes at the chronic course were the same as catalase activity of the same subgroup. In patients with exacerbated periodontitis, iron saturation of TF immediately increased in 1.08 times (p<0.05) and remained at the same level for 6 months. After 12 months, TF iron saturation decreased and the difference with the original data became insignificant (p>0.05).

The activity of serum CP in patients with initial periodontitis was increased in all examined: at chronic course – slightly (in 1.07 times; p>0.05), at exacerbated – significantly (in 1.12 times; p<0.05). Thanks to our measures, it has significantly decreased. The parameters were lower than in the healthy persons in both subgroups immediately and six months after treatment. A year later, the activity of CP increased in all patients, and the difference with the original data became insignificant (p>0.05).

Catalase activity was significantly higher immediately and six months after treatment in patients with chronic
### Table I. Changes in lipid peroxidation in the serum of patients with periodontitis after complex treatment (M±m)

<table>
<thead>
<tr>
<th>Exponents</th>
<th>Healthy</th>
<th>Periodontitis chronic course</th>
<th>Periodontitis exacerbated course</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>before treatment</td>
<td>after treatment</td>
</tr>
<tr>
<td>conjugates, (conventional units in 1 ml of plasma)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=17</td>
<td>0,786±0,03</td>
<td>n=18 1,026±0,08</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>n=20</td>
<td>3,17±0,12</td>
<td>n=20 3,11±0,07</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>conjugates, (conventional units in 1 ml of plasma)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=17</td>
<td>1,14±0,01</td>
<td>n=19 1,14±0,01</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>n=20</td>
<td>3,17±0,12</td>
<td>n=20 3,11±0,07</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>malonic dialdehyde, (nmol / ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=17</td>
<td>0,786±0,03</td>
<td>n=16 1,16±0,12</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>n=20</td>
<td>3,17±0,12</td>
<td>n=20 3,18±0,15</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

**Note.** Here and in Table II the probability of a difference of exponents is specified: p<0.05 due to exponents of healthy; p<0.05 due to data before treatment; p<0.05 due to data after treatment; p<0.05 due to data 6 months after treatment.

periodontitis (p<0.05). The result of treatment became even better in the case of an exacerbated course and after 6 months the norm was reached. After 12 months, catalase activity was in 1.12 times higher than before treatment (p<0.05).

The iron saturation of serum TF at periodontitis of the first degree was reduced in a case of chronic course in 1.13 times, and in the case of exacerbated - in 1.15 (p<0.001). It has contributed to increasing it in 1.15 times (p<0.001) after complex measures made by us in a case of chronic course, with exceeding the norm. Subsequently, there was some decrease in the level of iron saturation of TF, but the obtained values remained significantly different from the original data. (p<0.05).

Identical patterns were revealed in both subgroups of patients with periodontitis of the first degree according to the activity of CP in the serum: increasing before treatment in 1.14 and 1.16 times (p<0.005) and a significant decreasing immediately and 6 months after treatment in both subgroups, in particular in the case of an exacerbated course – in 1.15 and 1.14 times (p<0.005).

It is interesting to note that catalase activity in all patients with periodontitis of the II degree in all terms of observation was regulated successfully. Especially it grew after six months (p<0.01).

The effectiveness of our complex therapy was also manifested by the regulation of iron saturation of TF in serum. It was reduced in 1.20 times before treatment. (p<0.01) in both subgroups of patients with periodontitis of the II degree. This figure increased immediately after therapy in 1.15 times at the chronic course (p<0.001). The achieved result was maintained for six months (p=0.001) and changed little after a year (p<0.05). The iron saturation of transferrin increased immediately in 1.17 times (p<0.001) in the subgroup with an exacerbated course of periodontitis.

The highest level of CP activity was in the case of periodontitis of the II degree, and the difference with healthy was 1.26
REGULATION OF ANTIOXIDANT ENZYMES IN PATIENTS AFTER PERIODONTAL TREATMENT WITH NATURAL AGENTS

Table II. Dynamics of activity of antioxidant enzymes in the serum of patients with periodontitis under the influence of complex treatment (M±m)

<table>
<thead>
<tr>
<th>Exponents</th>
<th>Healthy</th>
<th>Periodontitis chronic course</th>
<th>Periodontitis exacerbated course</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>before treatment</td>
<td>after treatment</td>
</tr>
<tr>
<td>Catalase, (conventional units)</td>
<td>n=32 14,77±0,48</td>
<td>n=21 13,65±0,44</td>
<td>p&lt;0,05</td>
</tr>
<tr>
<td>Transferrin, (conventional units)</td>
<td>n=34 0,196±0,004</td>
<td>n=20 0,181±0,004</td>
<td>p&lt;0,01</td>
</tr>
<tr>
<td>Ceruloplasmin, (conventional units)</td>
<td>n=36 31,28±0,92</td>
<td>n=22 33,40±1,35</td>
<td>p&lt;0,05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>before treatment</td>
<td>after treatment</td>
</tr>
<tr>
<td>Catalase, (conventional units)</td>
<td>n=32 14,77±0,48</td>
<td>n=21 13,07±0,44</td>
<td>p&lt;0,05</td>
</tr>
<tr>
<td>Transferrin, (conventional units)</td>
<td>n=34 0,196±0,004</td>
<td>n=20 0,174±0,003</td>
<td>p&lt;0,01</td>
</tr>
<tr>
<td>Ceruloplasmin, (conventional units)</td>
<td>n=36 31,28±0,92</td>
<td>n=20 35,73±1,43</td>
<td>p&lt;0,05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>before treatment</td>
<td>after treatment</td>
</tr>
<tr>
<td>Catalase, (conventional units)</td>
<td>n=32 14,77±0,48</td>
<td>n=20 12,06±0,65</td>
<td>p&lt;0,05</td>
</tr>
<tr>
<td>Transferrin, (conventional units)</td>
<td>n=34 0,196±0,004</td>
<td>n=20 0,163±0,005</td>
<td>p&lt;0,01</td>
</tr>
<tr>
<td>Ceruloplasmin, (conventional units)</td>
<td>n=36 31,28±0,92</td>
<td>n=20 39,31±2,42</td>
<td>p&lt;0,05</td>
</tr>
</tbody>
</table>

Note. See table I.

and 1.34 times (p1=0.005; p1<0.001) at chronic and exacerbated course, respectively. Our treatment measures helped to reduce it in patients with chronic periodontitis immediately and 6 months after treatment in 1.22 times (p<0.05). The activity of CP increased and differed little from baseline 12 months after treatment (p<0.05). Somewhat different patterns are observed in the case of an exacerbated course, namely: reducing the activity of CP immediately (in 1.24 times; p<0.01), some growth of it 6 months after treatment and the maintenance achieved during the year.
The studied parameters were processed using correlation analysis. We found that there were strong reliable direct correlations between the content of DC and MDA in the serum in all follow-up periods before and after treatment ($r>0.89-0.99; p<0.005-0.001$). It was revealed a direct correlation between catalase activity and TF iron saturation before treatment ($r>0.98; p<0.001$) and indirect correlation between catalase and CP activity ($r>-0.98; p<0.001$) and TF and CP ($r>-0.99; p<0.001$). Only one reliable correlation remained immediately, 6 and 12 months after treatment – between parameters of TF and CP ($r=-0.83; -0.94; -0.99; p<0.005-0.001$).

After analyzing the relationship between LP and AO protection, we see that before therapeutic measures they were closely related, namely: catalase – with DC ($r>-0.91; p<0.005$) and with MD ($r>-0.98; p<0.001$); TF – with DC ($r>-0.86; p<0.005$) and with MDA ($r>-0.95; p<0.005$), CP – with DC ($r>-0.86; p<0.005$) and with MDA ($r>-0.95; p<0.005$). The relationship between catalase and MDA held true immediately after treatment and six months later ($r>-0.83-0.86; p<0.005$). A year later, this relationship remained and a correlation between CP and MDA was added ($r>-0.85; p<0.005$) (table II).

**DISCUSSION**

Thus, our research confirmed the significant role in the pathogenesis of periodontitis disorders in the pro- and AO system. It was found that with the deepening of dystrophic-inflammatory processes in the periodontium increases the intensification of LP and depletes the AO defense system. This is manifested by increased levels of DC and MDA, and a decrease in catalase activity, iron saturation of TF and increased activity of CP in serum. Similar were obtained by other researchers in the serum and oral fluid of patients. [5, 11-18].

It was possible to normalize the detected disorders for a long time under the influence of the developed method of complex treatment and to achieve clinical stabilization of the disease using of the drug Spirulina platensis. The content of the DC and MDA significantly increases as well. These indicators decreased in all patients at all follow-up periods ($p<0.05-0.001$) under the influence of complex treatment and were close to normal during the year ($p>0.05$). Catalase activity and iron saturation of TF decrease and CP activity increases at the same time. Catalase activity and iron saturation of TF increased in all patients at all follow-up periods ($p<0.05-0.01; p<0.05-0.001$) as a result of therapy and differed slightly from data in healthy patients ($p>0.05$).

Their complete normalization lasted six months at the initial and first degrees. CP activity decreased and differed significantly from the baseline of six months in all groups ($p<0.05-0.005$). Clinical stabilization of periodontitis was achieved in all patients.

**REFERENCES**

ORGANIZATION OF ANTIOXIDANT ENZYMES IN PATIENTS AFTER PERIODONTAL TREATMENT WITH NATURAL AGENTS

The scientific effort was performed as part of two research works on a base of Ivano-Frankivsk National Medical University, namely: „Complex methods of diagnosis, prevention and treatment of dental diseases of the population of Ivano-Frankivsk region”, Registration Number 0103U001013, which was performed at own expense, and „Development of methods for diagnosis, treatment and prevention of dental diseases in the population living in environmentally unfavorable conditions”, Registration Number 0111U003681, which was performed at the request of the Ministry of Health of Ukraine and financed from the state budget of Ukraine.

ORCID and contributionship:
Halyna M. Melnychuk: 0000-0002-2611-9048 A-R,F
Hanna D. Semeniuk: 0000-0002-9368-782X R,D,E
Roxolana S. Kashivska: 0000-0002-2028-4616 R,D
Natalia I. Shovkova: 0000-0002-2248-6297 C,E
Nadiia S. Melnyk: 0000-0002-7593-7100 C,E

Conflict of interest:
The Authors declare no conflict of interest.