DYNAMICS OF ANTIOXIDANT ENZYME ACTIVITY AND MALONIC DIALDEHYDE LEVEL IN THE ORAL FLUID OF PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS AND RHEUMATOID ARTHRITIS WHO LIVE IN ECOLOGICALLY UNFAVORABLE AREA UNDER THE INFLUENCE OF COMPLEX TREATMENT

K.V. Kovalyshyn, M.M. Rozhko

Ivano-Frankivsk National Medical University, department of Dentistry Postgraduate Education, Ivano-Frankivsk, Ukraine,
ORCID ID: 0000-0002-6477-6663,
ORCID ID: 0000-0002-68762533,
e-mail: endodoc89@gmail.com

Abstract. Inflammatory-destructive changes in periodontal tissues are more common in patients with concomitant somatic diseases and due to adverse environmental factors. This necessitates the development of new treatments aimed at eliminating the etiopathogenetic factors of generalized periodontitis development.

The aim to study the indicators changes in antioxidant protection and malonic dialdehyde (MDA) levels in the oral fluid in patients with GP and rheumatoid arthritis (RA) who live in environmentally unfavorable areas and assessment of the dynamics of changes in the studied indicators in the oral fluid under the influence of comprehensive treatment.

Materials and methods. 105 patients with GP of the I-II degree of development were examined and treated, of which 70 patients were with RA. Depending on the ecological living conditions of patients with RA was divided into two groups: group I (n=35) – live in environmentally unfavorable areas; group II (n=35) – live in environmentally favorable areas. Group III (n=35) was formed by patients with GP without concomitant pathology. Each of the groups, depending on the severity of GP was divided into subgroups: A – GP of the I degree; B – GP of the II degree of severity. The control group consisted of 18 virtually healthy individuals with intact periodontium and preserved dentition (H). The state of the prooxidant system of the oral cavity was determined by the level of MDA. To study the state of the antioxidant defense system (AOS), the activity of superoxide dismutase (SOD) and catalase was determined.

Research results. Determining the level of MDA in oral fluid in all subgroups of patients with GP and RA and without concomitant pathology revealed an increase in this indicator before treatment. It increased most significantly in the IB subgroup to 1.19±0.02 nmol/l and in the IA subgroup to 1.16±0.01 nmol/l, compared with 0.34±0.17 nmol/l in healthy individuals. (*p<0.001, *p<0.001), which exceeded by 3.5 and 3.4 times, respectively, which may be due to the negative impact of the environment. During all follow-up periods after conducted treatment, the level of MDA decreased and most closely approached the rate of healthy individuals after 6 months. However, before treatment, depletion of the antioxidant system was detected in all subgroups, as evidenced by the weakening of the AOS. In particular, in the IB subgroup there is the greatest decrease in the activity of SOD to 35.6±0.56%, compared with the group of healthy individuals 49.06±0.03% (p<0.001) and catalase to 4.42±0.03 c.u along with healthy – 6.64±0.01 c.u (p<0.001). The activity of certain antioxidant protection enzymes tends to increase after a comprehensive treatment in all subgroups after 6 months. And in the IB subgroup the activity of SOD after 6 months was 44.77±0.35%, catalase 6.29±0.3 c.u.

Conclusions. As a result of complex treatment there was normalization of the AOS system, which is manifested by increased activity SOD and catalase in the oral fluid, as well as a decrease in MDA. This indicates a decrease in inflammation process and the effectiveness of our treatment and prevention complex on the antioxidant system, which provided remission GP in these patients.

Keywords: generalized periodontitis, rheumatoid arthritis, ecologically unfavorable areas, prooxidant-antioxidant system.

Introduction. Problem statement and analysis of recent research. Periodontal tissue disease, in particular generalized periodontitis (GP), remains an important problem in modern dentistry due to its high prevalence among the population. Periodontal tissues reflect the influence of negative factors of the external and internal environment, and disturbances of homeostasis of the human body [2, 4, 22]. Therefore, the study of pathological changes of this tissue complex in patients with RA who live in environmentally unfavorable areas is extremely important in the diagnosis and development of the treatment complex.

Oral fluid is one of the main factors influencing the condition of periodontal tissues and is an indicator of impaired resistance of oral tissues [2, 7]. Examination of the oral fluid is a non-invasive, easy to perform and safer method compared to the research of blood, and the results of the analysis of the chemical composition of saliva and blood plasma are a reflection of each other and may indicate a pathology.
In the pathogenesis of inflammatory-dystrophic processes in periodontal tissues, one of the main links is the strengthening of lipid peroxidation (LPO) and the weakening of AOS [5]. The most important components of anti-radical and anti-peroxide protection are enzymes that catalyze the reactions between active oxygen species, thereby decomposing hydroperoxides. These enzymes include superoxide dismutase, glutathione peroxidase, catalase. The main function of these enzymes is the neutralization of the superoxidation-radical (O₂⁻) and hydrogen peroxide (H₂O₂), which are formed as a result of the discharge of an unpaired electron from the mitochondrial chain during electron transfer. SOD disrupts O₂⁻ to hydrogen peroxide, which is reduced by catalase to water and atomic oxygen or glutathione peroxidase to water [6, 17].

Many scientific works have been devoted to the study of POL and AOS in periodontitis [10, 18, 19, 20]. A number of scientists have investigated that the activity of SOD in the oral fluid is reduced in GP, which is a sign of oxidative stress [9, 11, 14, 15]. Opposite data are provided by a number of researchers who explain the increase in the activity of this enzyme as the activation of the body's compensatory forces [4, 12]. According to scientists, catalase activity decreases [4, 7, 14, 15]. Pathological effects caused by concomitant somatic pathology and adverse effects of the environmental situation, exacerbates the depletion of the AOS, which leads to a violation of the protective functions of periodontal tissues and the development of the inflammatory process. That is why patients with GP and RA, who live in environmentally unfavorable areas, need a more detailed study of these indicators.

The aim to study the changes in the indicators of AOS and the level of MDA in the oral fluid in patients with GP and RA who live in environmentally unfavorable areas and to assess the dynamics of the studied indicators in the oral fluid under the influence of comprehensive treatment.

Materials and methods of research. 105 patients with GP of the I-II degree of development were examined and treated, of which 70 patients were with RA. The diagnosis of RA was established by a rheumatologist on the basis of clinical, laboratory and radiological data according to the unified clinical protocol "Rheumatoid Arthritis", approved by the Order of the Ministry of Health of Ukraine 11.04.2014 №263. Treatment prescribed by a rheumatologist consisted of the use of basic antirheumatic drugs and nonsteroidal anti-inflammatory drugs.

Patients with GP and RA were divided into two groups. From the residents of Kalush district of Ivano-Frankivsk region (this region has the status of a "zone of ecological emergency" [3, 13]) was formed I group of 35 patients with RA, who live in environmentally unfavorable areas. Group II included 35 patients with RA who live in environmentally favorable areas (Ivano-Frankivsk). The third group consisted of patients with GP, without concomitant somatic pathology, who live in an environmentally favorable area – 35 people. Group IV consisted of healthy people who had intact periodontium and preserved dentition and no somatic diseases – 18 people (I).

Each of the groups, depending on the severity of GP was divided into subgroups: A – GP of the I degree; B – GP of the II degree of severity. The diagnosis of periodontal tissue diseases was established according to the classification of MF Danylevsky (1994).

To obtain oral fluid, the methods developed by NF Levtsky were followed [8]. SOD activity was determined by the method of C Chevara [16], and quantitative determination of catalase was carried out by the method of Bach's Academy of Sciences and Zubkova's ST [1] (M ± m).

The proposed treatment included rehabilitation of the oral cavity, its professional hygiene with the training of hygienic skills. Topical treatment included: rinsing the mouth cavity with Furazol solution (3 times a day for 7 days); antimicrobial and anti-inflammatory drug "Hosital" – applications to the gums 2 times a day for 10 days. For general treatment, patients of all groups received the drug Osteogenon 1 tablet 2 times a day; course of treatment for 6 months. To improve antioxidant protection, the antioxidant "E 200-Zentiva" was prescribed 1 tablet 2 times a day, for 14 days in courses once in four months (a total of 3 courses a year).

For patients of group I in order to remove exotoxins and endotoxins prescribed enterosorbet "Enterosgel" 1 tablespoon 3 times a day for an hour and a half after meals and drugs, for 7 days, courses once a month.

The obtained results were statistically processed using computer programs Microsoft Excel and Statistica 7.

Research of the results. When determining the level of MDA before treatment in all subgroups, the growth of this indicator was found (Fig. 1). It increased most significantly in the IB subgroup to 1.19±0.02 nmol/l and in the IA subgroup to 1.16±0.01 nmol/l, compared with 0.34±0.17 nmol/l in healthy individuals. (*pIAH<0.001, *pIAA<0.001), which exceeded it by 3.5 and 3.4 times, respectively. In patients with GP and RA who lived in environmentally favorable areas, the indicator was in the IB subgroup 1.14±0.03 nmol/l, IIA subgroup 1.11±0.07 nmol/l and that 3.35 times and 3.26 times significantly higher than healthy (*pIBA-H<0.001, *pRAA-H<0.001). In patients with GP without concomitant pathology, who lived in ecologically clean areas, this indicator was slightly lower and amounted to 1.01±0.02 nmol/l in subgroup IIB, 0.89±0.02 nmol/l in subgroup IIA and, which is 2.97 and 2.62 times significantly more than this indicator in the group of healthy individuals (*pIBA-H<0.001, *pRAA-H<0.001). Thus, we can confirm that the unfavorable environmental situation and somatic pathology affect the level of MDA in saliva in patients with GP. The highest rate in patients with GP of the II degree in all subgroups is due to the severity of the course of GP.

Analyzing our results of the level of MDA after treatment, we note a decrease in all subgroups. In particular, after 3 months it decreased significantly 1.68 times, after 6 months 2.57 times and after 12 months 2.23 times in the IA subgroup and 1.7, 2.38, 2.12 times after 3, 6 and 12 months, respectively, in the IB subgroup (*p<0.001).
In group II we got a somewhat similar picture, in particular in subgroup IIA the level of MDA significantly decreased by 1.63, 2.64, 2.52 times and in group IIB – by 1.54, 2.42, 2.32 times after 3, 6 and 12 months (*p<0.001).

In group III it was possible to stabilize this indicator the most and brought its value closer to the indicators in healthy individuals, as patients were not burdened by concomitant pathology and adverse effects of the environmental situation. In particular, the level of MDA decreased significantly in subgroup IIIA – 1.93, 2.54, 2.61 times and in IIB – 2.06, 2.42 and 2.4 times after 3, 6 and 12 months, respectively (*p<0.001).

The state of the antioxidant system was assessed by the activity of SOD enzymes and catalase in the oral fluid, which are presented in Fig.2 and Fig.3.
As can be seen from Fig. 2, in patients with GP of the I and II stage of development before treatment there is a significant decrease in the activity of SOD in the oral fluid in all subgroups. The greatest activity of SOD significantly decreases in groups I and II to the indicators 36.07±0.71% in subgroup IA, to 35.6±0.56% in subgroup IB, to 37.14±0.77% in subgroup IIA and up to 36.97±0.28% in the IIB subgroup, compared with the group of healthy individuals 49.06±0.03% (*p<0.001, *p<0.001, *p<0.001, *p<0.001). In group III, higher indicators of SOD activity were observed, in particular in subgroup IIIA – 42.13±0.23%, subgroup IIIB – 40.83±0.23% (*p<0.001, *p<0.001).

After conducted treatment after 3 months, the activity of SOD in the IA subgroup significantly increased in 1.27 times, after 6 months in 1.3 times and after 12 months in 1.25 times (*p<0.001). In the IB subgroup, it increased significantly in 1.21, 1.26, and 1.21 times after 3, 6, and 12 months, respectively (*p<0.001). A similar picture was observed in group II. SOD activity significantly increased in subgroup IIA in 1.29, 1.31, 1.26 times and in subgroup IIB in 1.25, 1.31, 1.26 times after 3, 6 and 12 months, respectively (*p<0.001). In subgroup IIIA, SOD activity significantly increased on average by 1.16 times, and in IIBB by 1.19 times during treatment (*p<0.001). According to the obtained results, it can be seen that due to the treatment, it was possible to stabilize the activity of SOD to the greatest extent after 6 months of all groups.

However, the obtained results also indicate a decrease in catalase activity in the oral fluid in all subgroups of patients before treatment (Fig. 3).

As can be seen from Fig. 3, the activity of catalase is significantly reduced to 4.6±0.07 c.u. in IA subgroup, to 4.42±0.03 c.u. in IB subgroup, to 4.81±0.07 c.u. in subgroup IIA and up to 4.54±0.03 c.u. in subgroup IIB, compared with 6.64±0.01 c.u. in the group of healthy individuals (*p<0.001, *p<0.001, *p<0.001, *p<0.001). In group III there was an increase in catalase activity, in particular in group IIIA is 5.71±0.43 c.u., in group IIB – 5.59±0.32 c.u (*p<0.001, *p<0.001).

Subsequently, under the influence of complex treatment, there is a normalization of catalase activity. In particular, in the IA subgroup the enzyme activity significantly increased in 1.33 times, after 6 months in 1.4 times and after 12 months in 1.36 times (*p<0.001). In the IB subgroup, catalase activity significantly increased by 1.28, 1.42, and 1.36 times after 3, 6, and 12 months, respectively (*p<0.001). A very similar picture was observed in group II. In subgroup IIA 1.3, 1.35, 1.33 times and in subgroup IIB 1.33, 1.4, 1.36 times significantly increased enzyme activity after 3, 6 and 12 months, respectively (*p<0.001). In subgroup IIIA – in 1.13, 1.16, 1.16 times, and in subgroup IIIB – in 1.17, 1.18, 1.15 times during treatment at different observation times (*p<0.001).

As can be seen from Fig. 2, in patients with GP of the I and II stage of development before treatment there is a significant decrease in the activity of SOD in the oral fluid in all subgroups. The greatest activity of SOD significantly decreases in groups I and II to the indicators 36.07±0.71% in subgroup IA, to 35.6±0.56% in subgroup IB, to 37.14±0.77% in subgroup IIA and up to 36.97±0.28% in the IIB subgroup, compared with the group of healthy individuals 49.06±0.03% (*p<0.001, *p<0.001, *p<0.001, *p<0.001). In group III, higher indicators of SOD activity were observed, in particular in subgroup IIIA – 42.13±0.23%, subgroup IIIB – 40.83±0.23% (*p<0.001, *p<0.001).

After conducted treatment after 3 months, the activity of SOD in the IA subgroup significantly increased in 1.27 times, after 6 months in 1.3 times and after 12 months in 1.25 times (*p<0.001). In the IB subgroup, it increased significantly in 1.21, 1.26, and 1.21 times after 3, 6, and 12 months, respectively (*p<0.001). A similar picture was observed in group II. SOD activity significantly increased in subgroup IIA in 1.29, 1.31, 1.26 times and in subgroup IIB in 1.25, 1.31, 1.26 times after 3, 6 and 12 months, respectively (*p<0.001). In subgroup IIIA, SOD activity significantly increased on average by 1.16 times, and in IIBB by 1.19 times during treatment (*p<0.001). According to the obtained results, it can be seen that due to the treatment, it was possible to stabilize the activity of SOD to the greatest extent after 6 months of all groups.

Thus, our research has shown that the course of GP of the I and II degree of development in the studied groups is accompanied by an imbalance of the prooxidant-antioxidant system of the oral cavity. However, it was found that in patients with GP and RA, who live in anthropogenically loaded areas, which leads to the accumulation of active oxygen species, the condition of the periodontium deteriorates, especially in patients with IB subgroup, which is confirmed by a significant increase in MDA and manifested by a decrease in the activity of the main antioxidant enzyme SOD, which catalyzes the dismutation of superoxide anions-radicals. The activity of a specific and powerful antioxidant barrier of the second line of defense, the enzyme catalase, is further reduced,
which indicates a weakening of the protection of oral tissues from the accumulation of reactive oxygen species.

In patients of group III, all the researched indicators were closest to the results of healthy, compared with patients of groups I and II, which indicates the negative impact of concomitant somatic pathology and adverse environmental situation in these groups.

Obtained results after conducted comprehensive treatment indicate the stabilization of the prooxidant-antioxidant system of the oral cavity in patients with GP of all subgroups and can be used as an objective criterion for assessing the depth of the pathological process in periodontal tissues and justify the need for inclusion in comprehensive treatment of patients with GP and RA of drugs with antioxidant action for correction of the established changes.

Discussion of the research. Summarizing the obtained results of the research of POL on the level of MDA and the activity of enzymes AOS, SOD and catalase before treatment revealed the greatest violation of these two systems in patients with GP and RA, who live in anthropogenically loaded areas. In particular, in patients with GP of the II degree of severity of the IB subgroup there is a significant increase in MDA – 1.19±0.02 nmol/ml, and in the IA subgroup – 1.16±0.01 nmol/ml, which slightly exceeded the results of this indicator in researches TV Pavlyuk – 1.02±0.09 nmol/ml [21]. As a result, a decrease in the activity of SOD in patients of I group in GP of the I and II degree of severity. In particular, in patients with GP of the II degree with RA in the IB subgroup, the enzyme activity decreases to 35.6±0.56%, and in patients with GP of the I degree with RA in the IA subgroup – 36.07±0.71%. Slightly lower indicators of SOD activity in GP of the I-II degree revealed by OL Lychkovska – 26.35±0.95% in GP of the I degree, and 22.39±1.56% in GP of the II degree [9], and TV Pavlyuk diagnosed much lower results of SOD activity – 20.64±0.82% [21]. Catalase activity in all patients with GP was also reduced, but the lowest rates were also diagnosed in patients with IB subgroup – 4.42±0.03 c.u. The results obtained by us are consistent with the results of HD Semenyuk 4.69 c.u. [14, 15].

At different times of observation after conducted complex therapy in patients with GP and RA who live in environmentally unfavorable areas, the antioxidant-prooxidant imbalance changes in the direction of a decrease in prooxidants, which is confirmed by a decrease in the level of MDA and an increase in antioxidant protection, which is manifested by an increase in the activity of SOD and catalase enzymes. Approximation of these indicators to the control group is observed after 6 months. In particular, the decrease in the level of MDA to 0.45±0.02 nmol/ml in the IA subgroup and to 0.5±0.01 nmol/ml in the IB subgroup against the background of increasing activity of enzymes AOS: SOD – 46.89±0.66% and catalase – 6.44±0.05 c.u in IA subgroup and SOD – 44.77±0.35% and catalase – 6.29±0.03 c.u in the IB subgroup.

Similar dynamics of the researched parameters after treatment was observed in the results of TV Pavlyuk and HD Semenyuk [14, 21, 23].

Conclusions. The results of conducted biochemical researches of oral fluid in patients with GP and RA who live in environmentally unfavorable areas, showed a marked weakening of AOS, which is manifested by a decrease in the activity of SOD enzymes, catalase and increased levels of MDA in these patients. The differences between the results of the examination in patients of groups I, II and III indicate a significant progression of the pathological process in patients of group I.

After the conducted complex treatment, there is a normalization of the antioxidant defense system, which is manifested in increased activity in the oral fluid of SOD and catalase, as well as a decrease in MDA, which indicates a decrease in inflammation and the effectiveness of our treatment and prevention complex on the antioxidant system which provided remission of GP in these patients.

Prospects for further researches. Further research will be aimed at in-depth study of periodontal tissue damage caused by environmental toxicity in patients with GP of the I, II degree with RA. Implementation of the proposed treatment and prevention complex for the normalization of oral cavity AOS.

References:

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ДИНАМІКА АКТИВНОСТІ ФЕРМЕНТІВ АНТИОКСИДАНТНОГО ЗАХИСТУ ТА РІВНЯ МАЛОНОВОГО ДІАЛЬДЕГІДУ РОТОВОЇ РІДИНІ У ХВОРИХ НА ГЕНЕРАЛІЗОВАНИЙ ПАРОДОНТИТ З РЕВМАТОЇДНИМ АРТРИТОМ, ЯКІ ПРОЖИВАЮТЬ НА ЕКОЛОГІЧНО НЕСПРИЯТЛИВИХ ТЕРИТОРІЯХ, ПІД ВПЛИВОМ КОМПЛЕКСНОГО ЛІКУВАННЯ

Х.В. Ковалєнко, М.М. Рожко

Івано-Франківський національний медичний університет, кафедра стоматології післядипломної освіти, м. Івано-Франківськ, Україна,

ORCID ID: 0000-0002-6477-6663,
ORCID ID: 0000-0002-86782533,
email: endodoc89@gmail.com

Резюме. Розробку нового комплексу лікування спрямовано на усунення етіопатогенетичних чинників розвитку генералізованого пародонтиту з ревматоїдним артритом.

Мета: вивчення змін показників антиоксидантного захисту та рівня малонового діальдегіду (МДА) у ротовій рідині у хворих на ГП з ревматоїдним артритом (РА), які проживають на екологічно несприятливих територіях, та оцінка їх динаміки під впливом проведеного комплексного лікування.

Матеріали та методи. Обстежено та проліковано 105 хворих на ГП І ступеня важкості, з яких 70 хворих на RA, яких поділено: І група (n=35) – проживають на екологічно сприятливих територіях; ІІ група (n=35) – екологічно сприятливих територіях, ІІІ група (n=35) – хворі на ГП без супутньої патології. Групи поділено на підгрупи: А – ГП І ступені; Б – ГП ІІ ступені важкості. 18 практично здорових осіб – контрольна група. Визначали рівень МДА, активність супероксиддисмутази (СОД) та каталази.

Результати дослідження. В ІІ підгрупі спостерігається найбільше зростання МДА у ротовій рідині до 1,16±0,01 нмоль/л та в ІА підгрупі до 1,16±0,01 нмоль/л, проти показника у здорових осіб 0,34±0,17 нмоль/л (*рв.<0,001, *рв.<0,001), зниження активності СОД в ІБ підгрупі до 35,60±0,56 %, каталази до 4,22±0,3 у.оз. Після проведеного лікування рівень МДА знижується, активність ферментів зростає у всіх підгрупах, досягаючи 6 місяців. Зокрема в ІБ підгрупі рівень МДА – 0,5±0,01 нмоль/л, активність СОД – 44,77±0,35 %, каталази 6,29±0,03 у.оз.

Висновки. Після проведеного комплексного лікування спостерігається нормалізація систем ПОЛ/АОС, що свідчить про ефективність впливу розробленого нами лікувально-профілактичного комплексу на антиоксидантну систему, що забезпечило ремісію ГП у даних хворих.

Ключові слова: генералізований пародонтит, ревматоїдний артрит, екологічно несприятливі території, прооксидантно-антioxidантна система.
Динамика активности ферментов антиоксидантной защиты и уровня малонового диальдегида ротовой жидкости у больных генерализованным пародонтитом с ревматоидным артритом, проживающих на экологически неблагоприятных территориях под влиянием комплексного лечения

Х.В. Ковалишин, М.М. Рожко

Ивано-Франковский национальный медицинский университет, кафедра стоматологии послепроизводственного образования, г. Ивано-Франковск, Украина,
ORCID ID: 0000-0003-273612609,
ORCID ID: 0000-0002-68762533,
e-mail: endodoc89@gmail.com

Резюме. Разработку нового комплекса лечения направлено на устранение этиопатогенетических факторов развития генерализованного пародонтита с ревматоидным артритом.

Цель: изучение изменений показателей антиоксидантной защиты и уровня малонового диальдегида (МДА) в ротовой жидкости у больных ГП с ревматоидным артритом (РА), проживающих на экологически неблагоприятных территориях и оценка их динамики под влиянием проведенного комплексного лечения.

Материалы и методы. Обследовано и проанализировано 105 больных ГП I-И степени тяжести, из которых 70 больных РА, которых разделено: I группа (n=35) — проживают на экологически неблагоприятных территориях; II группа (n=35) — экологически благоприятных территориях. Группа III (n=35) — больные ГП без сопутствующей патологии. Группы разделены на подгруппы: А — ГП I степени; Б — ГП II степени тяжести. 18 практически здоровых лиц — контрольная группа. Определяли уровень МДА, активность супероксиддисмутазы (СОД) и каталазы.

Результаты исследования. В IБ подгруппе наблюдается наибольший рост МДА в ротовой жидкости до 1,19±0,02 нмоль/л и в IA подгруппе до 1,16±0,01 нмоль/л, против показателя у здоровых лиц 0,34±0,17 нмоль/л (*p_{IB}-З<0,001, *p_{IA}-З<0,001), снижение активности СОД в IB подгруппе до 35,6±0,56%, каталазы до 4,42±0,03 у.е. После проведенного лечения уровень МДА снижался, активность ферментов росла во всех подгруппах, максимально через 6 месяцев. В частности в IB подгруппе уровень МДА — 0,5±0,01 нмоль/л, активность СОД — 44,77±0,35%, каталазы 6,29±0,03 у.е.

Выводы. После проведенного комплексного лечения наблюдается нормализация системы ПОЛ/АОЗ, что свидетельствует об эффективности влияния разработанного нами лечебно-профилактического комплекса на антиоксидантную систему, что обеспечило ремиссию ГП у данных больных.

Ключевые слова: генерализованный пародонтит, ревматоидный артрит, экологически неблагоприятные территории, прооксидантно-антиоксидантная система.

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