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The assessment of the status of the prooxidant-antioxidant system in patients with chronic generalized periodontitis in the post-covid period

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Resume. The article presents the results of the study of the intensity of free radical processes (FRP) and the activity of anti-oxidant systems (AOS) in the blood serum of people who were infected with the Sars-Cov-2 virus. The study of the initial level of TBC-active products and the increase in their concentration in the oral fluid of the research group, it showed that their value was significantly higher compared to the indicators of the representatives of the control group, which indicates an increase in lipid peroxidation. It was established that the catalase index and SOD activity of the salivary glands are significantly lower in the infected persons compared to similar indicators of the control group, which are due to long-term stress exposure, high emotional stress and depressive states of the patients and indicates the exhaustion of the antioxidant system. At the same time, there is an increase in such enzymes as glutathione peroxidase and glutathione reductase. It was found that the intensity of free radical processes and the imbalance of antioxidant systems increases with the deepening of immunodeficiency and with the progression of periodontal pathology.

Key words: Sars-Cov-2 virus, generalized periodontitis, peroxidation, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase.

Introduction

An important problem of modern dentistry remains the disease of periodontal tissues, in particular generalized periodontitis, which, according to the results of epidemiological studies, affects from 92 to 98 % over the age of 40 of the population of different regions of Ukraine [1, 9, 12]. Unfortunately, the risk factors for the occurrence of inflammatory diseases increase every year, not only in the elderly, but also in young people [3, 4, 6]. The activation of lipid peroxidation processes is a universal mechanism of tissue stress damage. Although oxidative stress appears to have a local oral origin, it is not currently understood whether it is caused by an excess of reactive oxygen species due to inflammation in the oral cavity or by insufficient antioxidants and depletion of AOS. In a number of works, a connection was found between the increased level of TBA-AP and the deterioration of the periodontal condition in the saliva of adults. It is noted that generalized periodontitis occurs much more often in people who have problems with the gastrointestinal tract or have been ill with viral infections [2, 13, 15, 16].

The outbreak of coronavirus disease 2019 (COVID-19) was first fixed in mid-december 2019 in China. Today, the coronavirus that causes severe acute respiratory syndrome (SARS-CoV-2) has affected the population of the whole world. According to Johns Hopkins University

(Baltimore, USA), from January 2020 to January 2022, 3,885,416 cases of coronavirus were fixed in Ukraine, and 107,831,598 confirmed cases were detected in Europe (figures updated: on January 10, 2022, 12:43 GMT +2) [14]. In many cases, infection with the coronavirus is characterized by damage to the bronchial architectonics of the lungs, the appearance of specific infiltrates and destruction, microcirculation disorders in the area of inflammation, and lung fibrosis. As a result, conditions are created in the patient's body for the development and growth of a hypoxic state, which contributes to the activation of lipid peroxidation [7]. In addition, in the case of viral damage in such patients is observed an increase in the number of opportunistic and anaerobic periodontopathogenic agents, which leads to a violation of the microbiosis of the gingival grooves and periodontal pockets [8, 10]. At the same time, the prerequisites for the emergence and development of inflammatory and dystrophic changes of the periodontium are created by unsatisfactory hygiene, which leads to an increase in the amount of dental deposits [11]. As a result of the decrease in local resistance and hypoxia, inflammatory processes deepen in the periodontium with the deterioration of oral hygiene. Oxidative stress also plays an important role in the pathogenesis of the 2019 coronavirus disease, which, by increasing the effects of cytokine shifts and strengthening blood coagulation and hypoxia, it causes tissue

damage and organ failure. The model of pathogenesis goes with the primary lung injury, late tissue hypoxemia and mitochondrial dysfunction, and profound changes in the antioxidant defense system [7]. Numerous studies indicate that an increase in the production of free radicals is registered against the background of a decrease in the antioxidant activity of the protective mechanisms of cells in patients with a severe course of the coronavirus disease. This is confirmed, in particular, by research data in Shanghai (China) during the 2019 pandemic. In particular, scientists have registered a significant increase in the activity of glutathione reductase in 40% of 198 examined patients with COVID-19, which was considered as a manifestation of an imbalance in the antioxidant defense system [17]. Thrombocytopenia was registered in 24 % of cases of COVID-19 and anemia in 59 %, respectively [18]. In connection with the high prevalence of infection with the Sars-Cov-2 virus among the population in Ukraine and in the world, there was a need to assess the antioxidant status of such patients, with the aim of developing a new complex to eliminate inflammatory and destructive changes in periodontal tissues. As saliva is an interesting alternative diagnostic fluid of the body, which has a number of advantages over blood: non-invasive and simple collection and the related possibility of repeated sampling, oral fluid was used for the study. The aim of our study was to identify the intensity of free radical processes (FRP) and the activity of antioxidant systems (AOS) in the oral fluid of patients with chronic generalized periodontitis (CGP) who were infected with the Sars-Cov-2 virus in the post-epidemic period.

Material and methods of research

200 patients with CGP were examined, who were treated for coronavirus infection in hospital conditions, of which 150 were diagnosed with post-acute pulmonary fibrosis and another 50 people with CGP, without aggravating concomitant pathology, who did not suffer from coronavirus infection. Clinical examination of patients was carried out on the basis of Ivano-Frankivsk Regional Clinical Hospital and Clinical Municipal Hospital 1. The diagnosis of the main disease was made by the inpatient physician in accordance with the clinical diagnostic criteria. Confirmation of Sars-Cov-2 virus infection was carried out by the PCR test method. The dental diagnosis was made according to clinical and laboratory criteria. The control group consisted of 40 practically healthy individuals with intact periodontium and preserved dentition. The age of the patients ranged from 45 to 60 years.

The state of the pro-oxidant system of the oral cavity was determined by the level of TBA-AP. To study the state of the antioxidant defense system, the enzymatic activity of superoxide dismutase (SOD) [5], catalase, glutathione peroxidase (GP) and glutathione reductase (GR) was determined. Standardized methods were used.

We carried out statistical processing of the data that we received with the help of the STATISTICA 7.0 program (StatSoft, USA), which are presented in the form of averages (M) with the error of representativeness (m). The Student's test (t) was used to determine the reliability of the difference between comparison groups. If the probability of error is $p < 0.05$, the difference was considered as probable.

Results and discussion

The analysis of the results of the dental examination of patients with GP in the Post-Covid period and those who were represented with Post-Covid pulmonary fibrosis allow to reveal certain features of the state of the periodontal tissues. It was established that patients with chronic generalized periodontitis had moderate inflammation of the gums; it was detected the pronounced recession of the gums and significant resorption of the bone tissue of the alveolar outgrowth. This testified that destructive processes are prevailing over the inflammatory phenomena.

It was found that with the progression of pathological changes in the periodontal tissues, the intensity of free radical processes increases, which is evidenced by the increase in the concentration of TBA-AP. At the same time, the level of TBA-AP changes significantly with the progression of periodontal pathology. The results that we obtained are presented in the table 1.

As can be seen from Table 1, the lowest content of TBA-AP in the oral fluid was observed in the 2nd group of patients who did not suffer from a corona virus infection, which exceeded by 1.2 times ($p < 0.05$) this indicator in the group of healthy individuals.

Regarding to patients who were treated from coronavirus infection in hospital conditions, this indicator was slightly higher: in the 3rd group, a significant increase in the level of TBA-AP was observed by 1.3 times ($p < 0.05$), in patients of the 4A and 4B subgroups – 1.32 times ($p < 0.05$), and 4C subgroups – 1.33 times ($p < 0.05$) relative to the group of healthy individuals. The data we received confirm the increase in oxidative stress in patients who have recovered from corona virus infection.

At the same time, a significant decrease in SOD activity was observed by the 2nd group by 1.2 times ($p < 0.05$), in the 3rd group by 1.29 times ($p < 0.05$), in the 4B subgroup – 1.44 times ($p < 0.05$) and 1.48 times ($p < 0.05$) in 4B and 4A subgroups (Table 2). A decrease in the activity of SOD leads to the growth of such secondary radicals as hydrogen peroxide and hydroxyl radical, which are neutralized by catalase and glutathione peroxidase. Accordingly, the activity of these enzymes was also determined by us.

As shown by our observations (Table 3), the activity of catalase is also significantly reduced in the oral fluid of patients in all studied groups in comparison with the group of healthy people. In particular, in patients with

GP of the 2nd group, catalase activity significantly decreases in 1.19 times ($p < 0.05$), in the 3rd group – by 1.16 times ($p < 0.05$).

The lowest values of catalase activity were found in the 4th group, lower than in the 2nd and 3rd groups. Thus, the activity of this enzyme decreases by 1.38 times ($p < 0.05$) in the 4A subgroup, by 1.44 times ($p < 0.05$) in the 4B subgroup, and by 1.46 times ($p < 0.05$). In the 4C subgroup, compared to the 1st group, which is obviously related to the progression of pathological changes in the periodontal tissues. Such results are consistent with the fact that we found a decrease

in the enzymatic activity of SOD, which indicates the depletion of the antioxidant protection system.

Table 4 presents the results of determination of GP in oral fluid. It can be seen that there is a significant increase in the activity of the enzyme in the groups of studied patients, in comparison with the group of healthy individuals. In the patients of the 2nd group, an increase in GP was found only by 1.16 times ($p < 0.05$) compared to the group of healthy individuals ($p < 0.05$), in the 4A subgroup – by 1.38 times ($p < 0.05$), in the 4B subgroup – by 1.21 times ($p < 0.05$) and in the 4C subgroup – as much as 1.5 times ($p < 0.05$) compared to the 1st group.

Table 1

The assessment of TBA-AP indicators in the oral fluid of patients with generalized periodontitis

Research indicators	1st group (healthy), n=40	2nd group, n=50	3rd group, n=50	4th group (n=150)		
				4A subgroup, n=50	4B subgroup, n=50	4C subgroup, n=50
TBA-AP, nmol/ml	3,46±0,07	4,16±0,05	4,48±0,06	4,57±0,02	4,55±0,04	4,59±0,04
	$P_{1-2} < 0,05$ $P_{1-3} < 0,05$ $P_{1-4A} < 0,05$ $P_{1-4B} < 0,05$ $P_{1-4C} < 0,05$	$P_{2-3} < 0,05$ $P_{2-4A} < 0,05$ $P_{2-4B} < 0,05$ $P_{2-4C} < 0,05$	$P_{1-3} < 0,05$ $P_{2-3} < 0,05$	$P_{1-4A} < 0,05$ $P_{2-4A} < 0,05$	$P_{1-4B} < 0,05$ $P_{2-4B} < 0,05$	$P_{1-4C} < 0,05$ $P_{2-4C} < 0,05$

Note: P_{1-2} – the level of differences between the 1st and 2nd groups; P_{1-3} – the level of differences between the 1st and 3rd groups; P_{1-4A} – the level of differences between the 1st group and the 4A subgroup; P_{1-4B} – the level of differences between the 1st group and the 4B subgroup; P_{1-4C} – the level of differences between the 1st group and the 4C subgroup; P_{2-3} – the level of differences between the 2nd and 3rd groups; P_{2-4A} – the level of differences between the 2nd group and the 4A subgroup; P_{2-4B} – the level of differences between the 2nd group and the 4B subgroup; P_{2-4C} – the level of differences between the 2nd group and the 4C subgroup;

Table 2

The assessment of the state of SOD indicators (%) in the oral fluid of patients with generalized periodontitis

Research indicators	1st group (healthy), n=40	2nd group, n=50	3rd group, n=50	4th group (n=150)		
				4A subgroup, n=50	4B subgroup, n=50	4C subgroup, n=50
SOD, %	48,95±0,07	40,62±0,18	37,74±0,32	34,07±0,35	32,82±0,49	33,14±0,47
	$P_{1-2} < 0,05$ $P_{1-3} < 0,05$ $P_{1-4A} < 0,05$ $P_{1-4B} < 0,05$ $P_{1-4C} < 0,05$	$P_{2-3} < 0,05$ $P_{2-4A} < 0,05$ $P_{2-4B} < 0,05$ $P_{2-4C} < 0,05$	$P_{1-3} < 0,05$ $P_{2-3} < 0,05$	$P_{1-4A} < 0,05$ $P_{2-4A} < 0,05$	$P_{1-4B} < 0,05$ $P_{2-4B} < 0,05$	$P_{1-4C} < 0,05$ $P_{2-4C} < 0,05$

Note: p_{1-2} – the level of differences between the 1st and 2nd groups; p_{1-3} – the level of differences between the 1st and 3rd groups; p_{1-4A} – the level of differences between the 1st group and the 4A subgroup; p_{1-4B} – the level of differences between the 1st group and the 4B subgroup; p_{1-4C} – the level of differences between the 1st group and the 4C subgroup; p_{2-3} – the level of differences between the 2nd and 3rd groups; p_{2-4A} – the level of differences between the 2nd group and the 4A subgroup; p_{2-4B} – the level of differences between the 2nd group and the 4B subgroup; p_{2-4C} – the level of differences between the 2nd group and the 4C subgroup;

Table 3

The assessment of catalase activity indicators (mcat/kg) in the oral fluid of patients with generalized periodontitis

Research indicators	1st group (healthy), n=40	2nd group, n=50	3rd group, n=50	4th group (n=150)		
				4A subgroup, n=50	4B subgroup, n=50	4C subgroup, n=50
Catalase, mkat/kg	6,64±0,01	5,56±0,02	5,74±0,02	4,81±0,02	4,61±0,02	4,54±0,02
	$P_{1-2} < 0,05$ $P_{1-3} < 0,05$ $P_{1-4A} < 0,05$ $P_{1-4B} < 0,05$ $P_{1-4C} < 0,05$	$P_{2-3} < 0,05$ $P_{2-4A} < 0,05$ $P_{2-4B} < 0,05$ $P_{2-4C} < 0,05$	$P_{1-3} < 0,05$ $P_{2-3} < 0,05$	$P_{1-4A} < 0,05$ $P_{2-4A} < 0,05$	$P_{1-4B} < 0,05$ $P_{2-4B} < 0,05$	$P_{1-4C} < 0,05$ $P_{2-4C} < 0,05$

Note: p_{1-2} – the level of differences between the 1st and 2nd groups; p_{1-3} – the level of differences between the 1st and 3rd groups; p_{1-4A} – the level of differences between the 1st group and the 4A subgroup; p_{1-4B} – the level of differences between the 1st group and the 4B subgroup; p_{1-4C} – the level of differences between the 1st group and the 4C subgroup; p_{2-3} – the level of differences between the 2nd and 3rd groups; p_{2-4A} – the level of differences between the 2nd group and the 4A subgroup; p_{2-4B} – the level of differences between the 2nd group and the 4B subgroup; p_{2-4C} – the level of differences between the 2nd group and the 4C subgroup;

Table 4

The assessment of glutathione peroxidase activity indicators ($\mu\text{mol}/(\text{min} \times \text{mg})$) in the oral fluid of patients with generalized periodontitis

Research indicators	1st group (healthy), n=40	2nd group, n=50	3rd group, n=50	4th group (n=150)		
				4A subgroup, n=50	4B subgroup, n=50	4C subgroup, n=50
Glutathione peroxidase, $\mu\text{mol}/(\text{min} \times \text{mg})$	0,18±0,01	0,21±0,01	0,24±0,01	0,25±0,02	0,27±0,01	0,27±0,01
	$P_{1-2} < 0,05$ $P_{1-3} < 0,05$ $P_{1-4A} < 0,05$ $P_{1-4B} < 0,05$ $P_{1-4C} < 0,05$	$P_{2-3} < 0,05$ $P_{2-4A} < 0,05$ $P_{2-4B} < 0,05$ $P_{2-4C} < 0,05$	$P_{1-3} < 0,05$ $P_{2-3} < 0,05$	$P_{1-4A} < 0,05$ $P_{2-4A} < 0,05$	$P_{1-4B} < 0,05$ $P_{2-4B} < 0,05$	$P_{1-4C} < 0,05$ $P_{2-4C} < 0,05$

Note: p_{1-2} – the level of differences between the 1st and 2nd groups; p_{1-3} – the level of differences between the 1st and 3rd groups; p_{1-4A} – the level of differences between the 1st group and the 4A subgroup; p_{1-4B} – the level of differences between the 1st group and the 4B subgroup; p_{1-4C} – the level of differences between the 1st group and the 4C subgroup; p_{2-3} – the level of differences between the 2nd and 3rd groups; p_{2-4A} – the level of differences between the 2nd group and the 4A subgroup; p_{2-4B} – the level of differences between the 2nd group and the 4B subgroup; p_{2-4C} – the level of differences between the 2nd group and the 4C subgroup;

The next stage was the determination of GR activity in the oral fluid, the obtained results are presented in Table 5.

As can be seen from Table 5, the activity of GR increased and significantly differed from the group of practically healthy persons by 1.3 times ($p < 0.05$) in the 2nd group, by 1.4 times ($p < 0.05$) in the 3rd subgroup, by 1.66 times ($p < 0.05$) in the 4A subgroup, 1.63 times ($p < 0.05$) in the 4B subgroup and 1.7 times ($p < 0.05$) – in subgroup 4C. However, the indicator of GR activity was unreliable in the 4th A subgroup, compared to the indicator in the 2nd group ($p > 0.05$).

The specified changes in these indicators indicate the development of destructive changes in the periodontal tissues, as GP and GR are the main enzymes that were involved in ensuring intracellular homeostasis.

Thus, after analyzing the results of the study of the indicated biochemical indicators of the oral fluid reliable changes of the studied parameters were established in accordance with the detected pathology of the periodontal tissues, with the progression of destructive changes of the periodontal tissues.

The assessment of glutathione reductase activity indicators ($\mu\text{mol}/(\text{min} \times \text{mg})$) in the oral fluid of patients with generalized periodontitis

Research indicators	1st group (healthy), n=40	2nd group, n=50	3rd group, n=50	4th group (n=150)		
				4A subgroup, n=50	4B subgroup, n=50	4C subgroup, (n=50)
Glutathione reductase,	0,27±0,01	0,35±0,01	0,38±0,01	0,45±0,01	0,44±0,01	0,46±0,01
	$P_{1-2} < 0,05$ $P_{1-3} < 0,05$ $P_{1-4A} < 0,05$ $P_{1-4B} < 0,05$ $P_{1-4C} < 0,05$	$P_{2-3} < 0,05$ $P_{2-4A} > 0,05$ $P_{2-4B} < 0,05$ $P_{2-4C} < 0,05$	$P_{1-3} < 0,05$ $P_{2-3} < 0,05$	$P_{1-4A} < 0,05$ $P_{2-4A} > 0,05$	$P_{1-4B} < 0,05$ $P_{2-4B} < 0,05$	$P_{1-4C} < 0,05$ $P_{2-4C} < 0,05$

Note: P_{1-2} – the level of differences between the 1st and 2nd groups; P_{1-3} – the level of differences between the 1st and 3rd groups; P_{1-4A} – the level of differences between the 1st group and the 4A subgroup; P_{1-4B} – the level of differences between the 1st group and the 4B subgroup; P_{1-4C} – the level of differences between the 1st group and the 4C subgroup; P_{2-3} – the level of differences between the 2nd and 3rd groups; P_{2-4A} – the level of differences between the 2nd group and the 4A subgroup; P_{2-4B} – the level of differences between the 2nd group and the 4B subgroup; P_{2-4C} – the level of differences between the 2nd group and the 4C subgroup;

Conclusion

Clinical studies conducted by us showed the connection of GP with markers of oxidative stress in saliva, such as TBA-AP and AOS, which protects the periodontium from the negative effects of AFOO. It has been shown that changes in the general state of the body, in particular post-acute pulmonary fibrosis, is caused by activation of lipid peroxidation, depletion of AOS and worsen the condition of periodontal tissues.

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Оцінка стану прооксидантно-антиоксидантної системи хворих на хронічний генералізований пародонтит у постковідному періоді

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Резюме. У статті наведені результати дослідження інтенсивності вільнорадикальних процесів (ВРП) і активності антиоксидантних систем (АОС) у сироватці крові осіб, які були інфіковані вірусом Sars-Cov-2. Вивчення початкового рівня ТБК-активних продуктів та приросту їх концентрації у ротовій рідині осіб дослідної групи показало достовірно вище їх значення порівняно із показниками представників групи контролю, що вказує на посилення пероксидного окислення ліпідів. Встановлено, що каталазний показник та активність СОД слинних залоз у інфікованих осіб достовірно нижчі порівняно з аналогічними показниками контрольної групи, що обумовлене тривалим стресорним впливом, високим емоційним напруженням та депресивними станами пацієнтів і вказує на виснаження антиоксидантної системи. Поряд з тим спостерігається зростання таких ферментів як глутатіонпероксидази та глутатіонредуктази. Виявлено, що інтенсивність вільно радикальних процесів та дисбаланс антиоксидантних систем наростає з поглибленням імунodefіциту та з прогресуванням пародонтопатології.

Ключові слова: вірус Sars-Cov-2, генералізований пародонтит, перекисне окислення, каталаза, супероксиддисмутаза, глутатіонпероксидаза, глутатіонредуктаза.

Оценка состояния прооксидантно-антиоксидантной системы у больных хроническим генерализованным пародонтитом в постковидный период

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Резюме. В статье представлены результаты исследования интенсивности свободнорадикальных процессов (СРП) и активности антиоксидантных систем (АОС) в сыворотке крови людей, инфицированных вирусом Sars-Cov-2. Изучение начального уровня ТБК-активных продуктов и повышение их концентрации в ротовой жидкости исследуемой группы показало, что их значение было достоверно выше по сравнению с показателями представителей контрольной группы, что свидетельствует об увеличении перекисного окисления липидов. Установлено, что каталазный индекс и активность СОД слюнных желез достоверно ниже у инфицированных лиц по сравнению с аналогичными показателями контрольной группы, которые обусловлены длительным стрессовым воздействием, высоким эмоциональным стрессовым и депрессивным состоянием больных и свидетельствует об истощении антиоксидантной системы. В то же время, наблюдается повышение таких ферментов, как глутатионпероксидаза и глутатионредуктаза. Установлено, что интенсивность свободнорадикальных процессов и дисбаланс антиоксидантных систем нарастает при углублении иммунодефицита и при прогрессировании патологии пародонта.

Ключевые слова: вирус Sars-Cov-2, генерализованный пародонтит, перекисная окисления, каталаза, супероксиддисмутаза, глутатионпероксидаза, глутатионредуктазы.

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