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MENOPAUSAL WOMEN WITH MODERATE AND ASYMPTOMATIC COVID-19: ANTIOXIDANT DEFENSE SYSTEM BIOMARKERS

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ABSTRACT

The aim. *The research was conducted to assess the total antioxidant and glutathione status, superoxide dismutase activity in menopausal women with moderate and asymptomatic COVID-19.*

Materials and methods. *Ninety two women 45 to 69 years old were divided into groups: women without COVID-19, not vaccinated, with no antibodies to SARS-CoV-2 (IgG) – control; women with moderate COVID-19 – main group; women with anti-SARS-CoV-2 IgG in blood but who deny any symptoms of COVID-19 in the last 12 months – asymptomatic COVID-19.*

Results. *A lower glutathione peroxidase (GPx), superoxide dismutase (SOD) activities and a higher glutathione reductase (GR) activity, glutathione S-transferase pi (GSTpi) concentrations were detected in the patients with moderate COVID-19 as compared to control. There were statistically lower oxidized glutathione (GSSG) levels, total antioxidant status (TAS) and higher reduced glutathione (GSH) levels, as well as GSH/GSSG ratio in the group with asymptomatic COVID-19 as compared to control. Significantly a lower GPx, SOD activities and a higher TAS, GR activity, GSTpi concentrations were detected in the patients with symptomatic COVID-19 as compared to group without clinical symptoms. ROC analysis shows the diagnostic significance of TAS (AUC = 0.714; p = 0.048), GSH (AUC = 0.714; p = 0.030), GSSG (AUC = 0.712; p = 0.031), GSH/GSSG (AUC = 0.837; p < 0.001) for group with asymptomatic COVID-19 compared with controls; TAS (AUC = 0.709; p = 0.020), SOD (AUC = 0.760; p < 0.001), GSH/GSSG (AUC = 0.658; p = 0.039), GPx (AUC = 0.774; p < 0.001), GSTpi (AUC = 0.864; p < 0.001) and GR (AUC = 0.871; p < 0.001) for group with moderate COVID-19 compared asympotmatic COVID-19.*

Conclusions. *Antioxidant defense system activity in menopausal women depends on the COVID-19 course.*

Key words: *total antioxidant status, glutathione, superoxide dismutase, COVID-19, menopause, women*

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СРЕДНЕТЯЖЁЛОЕ И БЕССИМПТОМНОЕ ТЕЧЕНИЕ COVID-19 У ЖЕНЩИН В МЕНОПАУЗЕ: БИОМАРКЕРЫ СИСТЕМЫ АНТИОКСИДАНТНОЙ ЗАЩИТЫ

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РЕЗЮМЕ

Цель. *Оценка общего антиоксидантного и глутатионового статуса, активности супероксиддисмутазы у женщин в менопаузе со среднетяжёлым и бессимптомным течением COVID-19.*

Материалы и методы. *Исследование включало 92 женщины в возрасте от 45 до 69 лет, которые были разделены на группы: женщины без COVID-19, не вакцинированные, без антител к SARS-CoV-2 (IgG) – контроль; женщины со среднетяжёлой формой заболевания COVID-19 – основная группа; женщины с IgG к SARS-CoV-2 в крови, но отрицающие какие-либо симптомы COVID-19 за последние 12 месяцев, – бессимптомное течение COVID-19.*

Результаты. *У пациентов со среднетяжёлым течением COVID-19 выявлена более низкая активность глутатионпероксидазы (GPx), супероксиддисмутазы (СОД) и более высокая активность глутатионредуктазы (GR), концентрации глутатион-S-трансферазы pi (GSTpi) по сравнению с контролем. В группе с бессимптомным течением COVID-19 наблюдались статистически более низкие уровни окисленной формы глутатиона (GSSG), общего антиоксидантного статуса (TAS, total antioxidant status) и более высокие уровни восстановленного глутатиона (GSH), а также соотношение GSH/GSSG по сравнению с контролем. У пациентов со среднетяжёлым течением COVID-19 выявлены статитсически значимо более низкие значения активности GPx, СОД и более высокие значения TAS, активности GR, GSTpi по сравнению с группой без клинических симптомов. ROC-анализ показал диагностическую значимость TAS (AUC (area under curve) – 0,714; p = 0,048), GSH (AUC = 0,714; p = 0,030), GSSG (AUC = 0,712; p = 0,031), GSH/GSSG (AUC = 0,837; p < 0,001) для группы с бессимптомным течением COVID-19 по сравнению с контрольной группой; TAS (AUC = 0,709; p = 0,020), СОД (AUC = 0,760; p < 0,001), GSH/GSSG (AUC = 0,658; p = 0,039), GPx (AUC = 0,774; p < 0,001), GSTpi (AUC = 0,864; p < 0,001) и GR (AUC = 0,871; p < 0,001) для группы со среднетяжёлым COVID-19 по сравнению с бессимптомным течением заболевания.*

Заключение. *Активность системы антиоксидантной защиты у женщин в менопаузе зависит от течения COVID-19.*

Ключевые слова: *общий антиоксидантный статус, глутатион, супероксиддисмутаза, COVID-19, менопауза, женщины*

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INTRODUCTION

During menopause, women begin to experience age-related neuroendocrine changes, accompanied by estrogen deficiency, which is considered as a condition for higher vulnerability to moderate and severe COVID-19 and subsequent complications [1]. It has been shown that menopausal women even 1 year after COVID-19, experience physical and emotional health problems, which may be due to endothelial dysfunction [2, 3]. One of the mechanisms for the development of endothelial dysfunction is oxidative stress that develops during SARS-CoV-2 virus infection [4–9].

The preservation of free radical balance in the body is realized by the antioxidant defense (AOD) system. One of the most significant components in the AOD system is the protein glutathione, which is involved in almost all stages of protection against oxidative stress [10]. The functions of glutathione can be realized through appropriate enzymes, which expand its properties to protect macromolecules. Thus, glutathione S-transferase (GST) catalyzes the conjugation reactions of glutathione with nonpolar substrates. Several different classes of GSTs are known, but the pi-class is the most studied due to its relationship with the development of human diseases [11]. Glutathione reductase (GR) is a reducing agent for oxidized glutathione (GSSG) to reduced glutathione (GSH), and glutathione peroxidase (GPx) restores free hydrogen peroxide and hydroperoxides [12]. Another important antioxidant is superoxide dismutase (SOD). This metal-containing enzyme inactivates the superoxide anion radical and is an active participant in the first line of defense against free radicals. There are several isoforms of the enzyme which differ in metal cofactors in the active center [13]. If the increase of reactive oxygen species level during COVID-19 is beyond doubt, the results of the antioxidant status studies of both the glutathione system and SOD activity during SARS-CoV-2 infection are ambiguous [4–8, 14, 15]. The reasons for the ambiguity of these results may be the lack of control groups in the studies, as well as the lack of consideration of age and gender, although these factors may influence the AOD system activity [16, 17].

THE AIM OF THE STUDY

An assessment of the total antioxidant and glutathione status, superoxide dismutase activity in menopausal women with moderate and asymptomatic COVID-19 in comparison to healthy ones.

MATERIALS AND METHODS

We employed a case-control research design (to explore differences between control and COVID-19) and a retrospective cohort study design (to examine of acute-phase parameters and post-COVID-19) in the current investigation. The study was carried out in the Scientific Centre for Family Health and Human Reproduction Problems with the informed consent of the participants as provided for by the Ethical norms of the Declaration of Helsinki of the World Medical Association (2013). The Research Protocol was approved by the Committee on Biomedical Ethics of this Scientific Centre (protocol No. 6.1 dated June 19, 2020).

The study included 94 women aged 45 to 69 years. In order to be selected into the main cohort, 64 women hospitalized in the Irkutsk Regional Infectious Clinical Hospital in the period from June 2020 to March 2021 with laboratory confirmed PCR test for the presence of SARS-CoV-2 virus and moderate course of COVID-19 accompanied by pneumonia were examined. Upon the patient's admission to the hospital, questionnaires and analysis of medical records, general clinical examination, and computed tomography were performed. One woman with 5.18 ng/ml anti-Mullerian hormone level was excluded from the main group (age – 58 ± 6.4; body mass index (BMI) – 30.03 ± 5.96 kg/m^2).

Thirty women who denied any symptoms of COVID-19 and had not been vaccinated in the past 12 months were examined to form a control group. The presence of COVID-19 IgG antibodies in blood was determined in all women, after which two groups were formed: without IgG (n = 17) and with IgG (n = 13). One woman with 14.61 ng/ml anti-Mullerian hormone level was excluded from the group without IgG; thus, 16 women formed the control group (age – 57 ± 6.24; BMI – 27.04 ± 3.69 kg/m^2). Thirteen women with IgG in their blood formed a separate group with asymptomatic COVID-19 (age – 54 ± 7.75; BMI – 28.63 ± 4.96 kg/m^2).

All study participants were examined by a general practitioner and cardiologist with calculation of BMI, measurement of blood pressure, body temperature, and electrocardiogram. In addition, all women noted amenorrhea or menstrual irregularities, consisting of stable fluctuations (from 7 days and above) in the duration of successive cycles. To exclude the presence of COVID-19 at the time of the study (control, asymptomatic COVID-19, post-COVID-19), an appropriate rapid test (RAPID BIO, Russia) was performed.

Exclusion criteria for all groups: regular menstrual cycle, use of hormone replacement therapy, anti-Mullerian hormone level > 1 ng/ml (for all participants). Exclusion criteria for the control group: exacerbation of chronic diseases, presence of IgG to SARS-CoV-2, positive result for the presence of the SARS-CoV-2; for the main group – absence of pneumonia.

Between 8.00 and 9.00 a. m., after 12 hours of overnight fasting, venous blood was sampled from the cubital vein into two tubes (with EDTA-K3 to obtain erythrocyte lysate and clot-activator to obtain serum). Whole venous blood was used immediately for general blood test determination. Then, samples were centrifuged for 10 min at 1.500 g. The remaining blood serum was collected in an Eppendorf tube and frozen. Erythrocytes were washed three times with 0.9% NaCl and centrifuged for 5 min at 1500 g after each wash. Afterwards, erythrocytes were resuspended in bi-distilled water at a 1:2 ratio, incubated for 10 min at 2 to 8 °C, then centrifuged at 1500 g for 5 min, the stroma was removed, the final 100-µL lysate output was mixed with 1.9 mL 0.9% NaCl and frozen. Serum for the determination of GR activity, TAS, anti-Mullerian hormone, GSTpi, IgG concentrations and hemolysate for the determination of GSH and GSSG levels, GPx and SOD activities, were stored at –40 °C until assaying.

The anti-Mullerian hormone (ng/ml) and IgG (BAU/ml) levels were determined on a MultiSkan ELX808 microplate reader (Biotek, Winooski, VT, USA) using the "Hema" test system and commercial "Vector-Best" kits, respectively. TAS (conventional units) was determined using commercial kits, RANSOD (Randox, Crumlin, UK). The measurements were carried out on a BTS-350 spectrofluorophotometer (Barcelona, Spain) at λ = 600 nm. GSH and GSSG levels (mmol/L) were detected on a spectrofluorophotometer "Fluorate 02-ABFF-T" (Russia) and performed at λ = 350 nm (excitation) and λ = 420 nm (absorption) [18]. GR and GP activities were assayed using "Randox" commercial kits (UK) on a "BTS-330" automatic photometer (Poland). Enzyme activity was expressed in units per 1 L of serum (for GR) or hemolysate (for GP) (U/L). GSTpi concentrations (ng/mL) was determined by enzyme immunoassay using "Cloud-Clone Corporation" kits (USA) on a MultiSkan ELX808 microplate reader (Biotek, Winooski, VT, USA) at λ = 450 nm. The activity of SOD in erythrocytes (equivalent units) was determined using commercial RANSOD kits (Randox Laboratories Ltd., Crumlin, UK) and a BTS-350 spectrofluorophotometer (Barcelona, Spain) as instructed by the manufacturer.

Statistical analysis

The obtained data were processed in STATISTICA 10 (StatSoft Inc., USA). A power calculation was not performed for this analysis. To determine the proximity to the normal law of distribution of quantitative signs, the visual-graphic method and the Kolmogorov – Smirnov test were used. The Fisher test was used to determine the equality of the general variances. Data on age and body mass index are presented as arithmetic mean ± standard deviation and analyzed using the parametric Student *t*-test. Antioxidant status parameters are presented as median [quartile 1 (Q1); quartile 3 (Q3)]. Analysis of intergroup differences for independent samples was carried out using the Kruskal – Wallis ANOVA by ranks and Median test, followed by post hoc (multiple comparisons) analysis using the Mann – Whitney *U*-test. The diagnostic value and optimal cut-off levels of studied parameters were determined based on the ROC analysis. All the differences were considered statistically significant at $p < 0.05$.

RESULTS

Table 1 summarizes the antioxidant defense system biomarkers in menopausal women with COVID-19 and control group. Significantly a lower GPx ($p = 0.021$), SOD ($p = 0.002$) activities and a higher GR activity ($p < 0.001$), GSTpi ($p < 0.001$) concentrations were detected in the patients with moderate COVID-19 as compared to control.

There were statistically lower GSSG ($p = 0.041$) levels, TAS ($p = 0.049$) and higher GSH ($p = 0.049$) levels, as well as GSH/GSSG ratio ($p = 0.001$) in the group with asymptomatic COVID-19 as compared to control.

Also, there were differences between COVID-19 patients and group with asymptomatic COVID-19. Significantly a lower GPx ($p = 0.001$), SOD ($p = 0.003$) activities and a higher TAS ($p = 0.017$), GR activity ($p < 0.001$), GSTpi ($p < 0.001$) concentrations were detected in the patients with symptomatic COVID-19 as compared to group without clinical symptoms.

In this study, a ROC analysis was carried out of the discriminatory abilities of antioxidant defense system biomarkers in the diagnosis of patients with asymptomatic COVID-19 and moderate COVID-19. For the ROC analysis, all indicators were examined to select the most significant ones. The usefulness of studied biomarkers in asymptomatic COVID-19 in comparison with those of the control group and moderate COVID-19 patients in comparison with asymptomatic COVID-19 are presented in Table 2 and Table 3 respectively.

ROC analysis shows the diagnostic significance of TAS (AUC = 0.714; $p = 0.048$), GSH (AUC = 0.714; $p = 0.030$), GSSG (AUC = 0.712; $p = 0.031$), GSH/GSSG (AUC = 0.837; $p < 0.001$) for group with asymptomatic COVID-19 compared with controls (Table 2).

When comparing the groups with moderate COVID-19 and asymptomatic COVID-19 the significance of the param-

TABLE 1

ANTIOXIDANT DEFENSE SYSTEM BIOMARKERS IN MENOPAUSAL WOMEN WITH COVID-19 AND CONTROL

Parameters	Control (*N* = 16)	Asymptomatic COVID-19 (*N* = 13)	COVID-19 (*N* = 63)	$P_{(ANOVA)}$
TAS, U/L	1.48 [1.29; 1.55]	1.27 [1.11; 1.44]*	1.45 [1.34; 1.6]^	0.007
SOD, U/L	1.58 [1.55; 1.58]	1.59 [1.57; 1.62]	1.24 [0.92; 1.59]*; ^	0.001
GSH, mmol/L	2.02 [1.7; 2.52]	2.45 [2.26; 3.08]*	2.35 [2.07; 2.73]	0.046
GSSG, mmol/L	2.1 [1.82; 2.37]	1.84 [1.62; 1.95]*	1.87 [1.62; 2.34]	0.072
GSH/GSSG	0.93 [0.85; 1.22]	1.44 [1.16; 1.78]*	1.26 [0.89; 1.51]	0.016
GPx, U/L	2126 [1820.5; 2412.5]	2377 [2056; 2558]	1804 [1321; 2162]*; ^	0.002
GSTpi, ng/mL	5.01 [3.67; 10.59]	6.02 [4.94; 7.85]	14.15 [11.52; 18.2]*; ^	< 0.001
GR, U/L	79.3 [70.75; 86.65]	73.3 [63.3; 79.6]	101.4 [86.1; 115.4]*; ^	< 0.001

Note. * – $p_U < 0.05$ as compared to control; ^ – $p_U < 0.05$ as compared to asymptomatic COVID-19.

TABLE 2

ROC ANALYSIS OF ANTIOXIDANT DEFENSE SYSTEM BIOMARKERS IN WOMEN WITH ASYMPTOMATIC COVID-19 IN COMPARISON WITH CONTROL GROUP

Parameter	AUC	*p*-value	Cut-off point	95% CI	Sensitivity	Specificity
TAS, U/L	0.714	0.048	≤ 1.46	0.517–0.865	84.62	68.75
SOD, U/L	0.625	0.258	> 1.58	0.427–0.797	53.85	75.00
GSH, mmol/L	0.714	0.030	> 1.89	0.517–0.865	92.31	50.00
GSSG, mmol/L	0.712	0.031	≤ 2.08	0.514–0.863	84.62	56.25
GSH/GSSG	0.837	< 0.001	> 0.947	0.653–0.947	100	56.25
GPx, U/L	0.620	0.266	> 2288	0.422–0.793	53.85	75.00
GSTpi, ng/mL	0.567	0.550	> 3.939	0.371–0.749	100	31.25
GR, U/L	0.630	0.227	≤ 79.6	0.432–0.800	76.92	50.00

Note. CI – confidence interval.

TABLE 3

ROC ANALYSIS OF ANTIOXIDANT DEFENSE SYSTEM BIOMARKERS IN WOMEN WITH MODERATE COVID-19 IN COMPARISON WITH ASYMPTOMATIC COVID-19

Parameter	AUC	*p*-value	Cut-off point	95% CI	Sensitivity	Specificity
TAS, U/L	0.709	0.020	> 1.27	0.593–0.807	87.30	53.85
SOD, U/L	0.760	< 0.001	≤ 1.48	0.643–0.854	70.18	100
GSH, mmol/L	0.618	0.169	≤ 2.19	0.500–0.728	39.68	84.62
GSSG, mmol/L	0.567	0.377	> 2.16	0.448–0.680	31.75	92.31
GSH/GSSG	0.658	0.039	≤ 0.941	0.540–0.763	31.75	100
GPx, U/L	0.774	< 0.001	≤ 1833	0.664–0.862	57.14	100
GSTpi, ng/mL	0.864	< 0.001	> 10.37	0.764–0.932	78.69	84.62
GR, U/L	0.871	< 0.001	> 86.1	0.775–0.937	74.60	92.31

Note. CI – confidence interval.

eters was identified for TAS (AUC = 0.709; $p = 0.020$), SOD (AUC = 0.760; $p < 0.001$), GSH/GSSG (AUC = 0.658; $p = 0.039$), GPx (AUC = 0.774; $p < 0.001$), GSTpi (AUC = 0.864; $p < 0.001$) and GR (AUC = 0.871; $p < 0.001$) (Table 3).

DISCUSSION

This investigation for the first time demonstrates antioxidant status assessment in menopausal women depending on the COVID-19 course. The SARS-CoV-2 virus appears to be an extreme factor for the organism. Hypoxia as a result of pneumonia leads to intensification of lipid peroxidation processes, the development of oxidative stress and damage to the endothelium. During infection, hidden regulatory and damaging mechanisms are "exposed" that determines the body's resistance to an emergency factor action. It may be the explanation, why not all infected people experience COVID-19 symptoms. In addition, menopause is a risk factor for the oxidative and carbonyl stress development [19, 20]. Antioxidant status changes in blood serum and cells of various organs have been shown in age-related estrogen deficiency [21–23] and during respiratory viral infections [24]. It may worsen free radical homeostasis during SARS-CoV-2 infection [4–9].

Changes in SOD activity in patients with COVID-19 are observed both in the direction of decreasing [15] and increasing [6] values. A decrease in SOD activity is observed in severe and critical COVID-19 compared with mild disease [15], and an increase in enzyme activity was detected in a study on elderly patients, regardless of the disease severity [6]. However, the results of a study on groups of patients depending on gender and age showed that women with COVID-19 over 36 years old have a lower SOD activity compared to women of 18–35 years. Probably the reason for this is an age-dependent decrease in the estrogen level in the female body, which can change the AOD system enzymes activity, including SOD. Thus, F. Bellanti et al. (2013) in their study showed a decrease in SOD mRNA expression with a simultaneous decrease in estrogen levels in women with surgical menopause, however after hormone replacement therapy, these indicators increased [25].

GSH production may be also decreased in response to COVID-19. This may be due to increased interleukin-6 and transforming growth factor-β levels, intracellular generation of free radicals and inhibition of BRCA1 [26]. The glutathione status study results showed lower GSH level in patients with COVID-19 compared to reference values [7], control [8] and does not differ between groups of patients in non-intensive and intensive care units [5]. There were no differences found in the GSH and GSSG levels, as well as their ratio and GST activity between groups with moderate and severe disease [4]. However, there are investigations that demonstrated differences in GSH level in patients with different severity COVID-19 [14]. In our study, we showed that glutathione system enzymes are activated in patients with moderate COVID-19. There is a lower GPx activity with a simultaneous higher GR activity and GSTpi level in these patients. Probably, it is response to the excessive formation of highly toxic lipid peroxidation products that are formed due to hypoxia during pneumonia. It has been shown that glutathione levels changes in lung inflammation [27]. Considering that the GPx kinetics is influenced by the GSH level [12], the decrease in this enzyme activity is most likely due to a decrease in GSH level according to the principle of direct feedback. H.K. Al-Hakeim et al. (2023) showed that decreased SpO_2 levels during the acute phase of COVID-19 significantly predict decreased GPx in Long COVID [28].

In addition, it is known that GPx activity can be induced by the hormone melatonin [29]. Serum melatonin levels in patients with COVID-19 are reduced due to disruption of its synthesis pathway by SARS-CoV-2 [30]. This also may be the reason for the decreased GPx activity in COVID-19 patients in our study. A higher GSTpi levels in our symptomatic COVID-19 group are required for timely detoxification processes by catalyzing the reactions of GSH conjugation with reactive oxygen and free radical oxidation products. Given the possible increased GSH consumption, timely GSSG restoration is necessary. This occurs when GR activity is increased, as we observed in our study. Considering the control levels of both GSH and GSSG in women with symptomatic COVID-19, it can be assumed that these enzymes activity is sufficient to maintain the thiol-disulfide equilibrium.

It is interesting to note that asymptomatic COVID-19 patients had higher GSH and GSH/GSSG levels than patients of the control group without any changes in enzymatic activity. Perhaps, this glutathione level determined the body's better resistance in response to SARS-CoV-2 virus infection, which prevented deterioration in the functioning of organs and systems and the clinical symptoms manifestation. Our results confirm the hypothesis of A. Polonikov (2020), who suggested that a higher initial GSH level determines the COVID-19 symptoms [31]. Moreover, GSH has inhibitory effects on the angiotensin-converting enzyme (ACE-2) activity and can decrease the production of reactive oxygen species via inhibition of ACE-2, leading to decreased nuclear factor-κB signaling, providing an avenue for decreased inflammation in SARS-CoV infected cells [32]. All this could be the reason for the absence of clinical symptoms in patients with higher GSH levels. We also identified a lower TAS level in this group compared to both control and moderate COVID-19. According to the studies conducted, there is no clear opinion on this parameter. Thus, A. Esmaeili-Nadimi et al. (2023) did not find any significant differences between groups with mild, moderate and severe disease [33], as well as when compared with controls [34], while the results of another study indicate a decrease TAS in patients in the intensive care unit [35]. In our study, we did not identify any differences in this indicator between moderate COVID-19 and controls. The fact that the indicator was lower in the group with asymptomatic disease may be because in these patients the AOD system functioning occurs at a sufficient level and its activation is not required. The results of an earlier study assessing life quality indicators in patients without COVID-19 clinical symptoms showed no differences with controls, however, scores for physical condition were higher in asymptomatic patients. Also, elevated high-density lipoprotein cholesterol level was noted as compared to control values [2]. These results and the present study data suggest the presence of a more advanced antioxidant system, better functioning of organs and systems, and a more adapted to respiratory virus infection immune system in patients who have asymptomatic COVID-19.

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Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

1. Prinelli F, Trevisan C, Noale M, Franchini M, Giacomelli A, Cori L, et al. Sex- and gender-related differences linked to SARS-CoV-2 infection among the participants in the web-based EPICOVID19 survey: The hormonal hypothesis. *Maturitas*. 2022; 158: 61-69. doi: 10.1016/j.maturitas.2021.11.015

2. Vyrupaeva EV, Semenova NV, Rychkova LV, Petrova AG, Darenskaya MA, Kolesnikov SI, et al. Assessment of the general condition and quality of life of women of post-reproductive age after asymptomatic COVID-19 and 12 months after moderate COVID-19. *Acta biomedica scientifica*. 2022; 7(5-1): 77-85. (In Russ.). doi: 10.29413/ABS.2022-7.5-1.9

3. Semenova NV, Kolesnikov SI, Vyrupaeva EV, Sholokhov LF, Rychkova LV, Petrova AG, et al. Thyroid status and TNF-alpha

in post-reproductive women with COVID-19 and 12 months after the disease. *Acta biomedica scientifica.* 2023; 8(2): 33-42. (In Russ.). doi: 10.29413/ABS.2023-8.2.4

4. Gadotti AC, Lipinski AL, Vasconcellos FT, Marqueze LF, Cunha EB, Campos AC, et al. Susceptibility of the patients infected with Sars-Cov2 to oxidative stress and possible interplay with severity of the disease. *Free Radic. Biol. Med.* 2021; 165: 184-190. doi: 10.1016/j.freeradbiomed.2021.01.044

5. Karkhanei B, Talebi Ghane E, Mehri F. Evaluation of oxidative stress level: Total antioxidant capacity, total oxidant status and glutathione activity in patients with COVID-19. *New Microbes New Infect.* 2021; 42: 100897. doi: 10.1016/j.nmni.2021.100897

6. Martín-Fernández M, Aller R, Heredia-Rodríguez M, Gómez-Sánchez E, Martínez-Paz P, Gonzalo-Benito H, et al. Lipid peroxidation as a hallmark of severity in COVID-19 patients. *Redox Biol.* 2021; 6(48): 102181. doi: 10.1016/j.redox.2021.102181

7. Pincemail J, Cavalier E, Charlier C, Cheramy-Bien JP, Brevers E, Courtois A, et al. Oxidative stress status in COVID-19 patients hospitalized in intensive care unit for severe pneumonia. A pilot study. *Antioxidants (Basel).* 2021; 10: 257. doi: 10.3390/antiox10020257

8. Lage SL, Amaral EP, Hilligan KL, Laidlaw E, Rupert A, Namasivayan S, et al. Persistent oxidative stress and inflammasome activation in $CD14^{high}CD16^{-}$ monocytes from COVID-19 patients. *Front Immunol.* 2022; 12: 799558. doi: 10.3389/fimmu.2021.799558

9. Horváth-Szalai Z, Jakabfi-Csepregi R, Szirmay B, Ragán D, Simon G, Kovács-Ábrahám Z, et al. Serum total antioxidant capacity (TAC) and TAC/Lymphocyte ratio as promising predictive markers in COVID-19. *Int J Mol Sci.* 2023; 24: 12935. doi: 10.3390/ijms241612935

10. Circu M, Aw TY. Glutathione and modulation of cell apoptosis. *Biochim Biophys Acta* 2012; 1823(10): 1767-1777. doi: 10.1016/j.bbamcr.2012.06.019

11. Dong SC, Sha HH, Xu XY, Hu TM, Lou R, Li H, et al. Glutathione S-transferase π: Potential role in antitumor therapy. *Drug Des Devel Ther.* 2018; 12: 3535-3547. doi: 10.2147/DDDT.S169833

12. Lankin VZ, Shumaev KB, Tikhaze AK, Kurganov BI. Influence of dicarbonyls on kinetic characteristics of glutathione peroxidase. *Dokl Biochem Biophys.* 2017; 475: 287-290. doi: 10.1134/S1607672917040123

13. Liu M, Sun X, Chen B, Dai R, Xi Z, Xu H. Insights into manganese superoxide dismutase and human diseases. *Int J Mol Sci.* 2022; 23(24): 15893. doi: 10.3390/ijms232415893

14. Kryukov EV, Ivanov AV, Karpov VO, Alexandrin VV, Dygai AM, Kruglova MP, et al. Association of low molecular weight plasma aminothiols with the severity of coronavirus disease 2019. *Oxid Med Cell Longev.* 2021; 2021: 9221693. doi: 10.1155/2021/9221693

15. Liao FL, Peng DH, Chen W, Hu HN, Tang P, Liu YY, et al. Evaluation of serum hepatic enzyme activities in different COVID-19 phenotypes. *J Med Virol.* 2021; 93(4): 2365-2373. doi: 10.1002/jmv.26729

16. Wang L, Ahn YJ, Asmis R. Sexual dimorphism in glutathione metabolism and glutathione-dependent responses. *Redox Biol.* 2020; 31: 101410. doi: 10.1016/j.redox.2019.101410

17. Semenova NV, Rychkova LV, Darenskaya MA, Kolesnikov SI, Nikitina OA, Petrova AG, et al. Superoxide dismutase activity in male and female patients of different age with moderate COVID-19. *Bull Exp Biol Med.* 2022; 173(1): 51-53. doi: 10.1007/s10517-022-05491-6

18. Nikitina OA, Darenskaya MA, Semenova NV, Kolesnikova LI. Antioxidant defense system: Regulation of metabolic processes, genetic determinants, methods of determination. *Siberian Scientific Medical Journal.* 2022; 42(3): 4-17. (In Russ.). doi: 10.18699/SSMJ20220301

19. Brichagina AS, Semenova NV, Kolesnikova LI. Age-related menopause and carbonyl stress. *Advances in Gerontology.* 2022; 12(4): 456-462. (In Russ.).

20. Semyonova NV. Oxidative stress and menopause (review of literature). *Acta biomedica scientifica.* 2014; 2(96): 120-125. (In Russ.).

21. Kolesnikova L, Semenova N, Madaeva I, Suturina L, Solodova E, Grebenkina L, et al. Antioxidant status in peri- and postmenopausal women. *Maturitas.* 2015; 81(1): 83-87.

22. Semenova NV, Brichagina AS, Madaeva IM, Kolesnikova LI. Enzymatic component of the glutathione system in Russian and Buryat women depends on the menopausal phase. *J Evol Biochem Physiol.* 2022; 58(4): 971-978. doi: 10.1134/S0022093022040032

23. Jin LY, Lv ZD, Wang K, Qian L, Song XX, Li XF, et al. Estradiol alleviates intervertebral disc degeneration through modulating the antioxidant enzymes and inhibiting autophagy in the model of menopause rats. *Oxid. Med. Cell Longev.* 2018; 2018: 7890291. doi: 10.1155/2018/7890291

24. Liu M, Chen F, Liu T, Chen F, Liu S, Yang J. The role of oxidative stress in influenza virus infection. *Microbes Infect.* 2017; 19(12): 580-586. doi: 10.1016/j.micinf.2017.08.008

25. Bellanti F, Matteo M, Rollo T, De Rosario F, Greco P, Vendemiale G, et al. Sex hormones modulate circulating antioxidant enzymes: Impact of estrogen therapy. *Redox Biol.* 2013; 1(1): 340-346. doi: 10.1016/j.redox.2013.05.003

26. Yegiazaryan A, Abnousian A, Alexander LJ, Badaoui A, Flaig B, Sheren N, et al. Recent developments in the understanding of immunity, pathogenesis and management of COVID-19. *Int J Mol Sci.* 2022; 23(16): 9297. doi: 10.3390/ijms23169297

27. Rahman I, Biswas SK, Jimenez LA, Torres M, Forman HJ. Glutathione, stress responses, and redox signaling in lung inflammation. *Antioxid Redox Signal.* 2005; 7(1-2): 42-59. doi: 10.1089/ars.2005.7.42

28. Al-Hakeim HK, Al-Rubaye HT, Al-Hadrawi DS, Almulla AF, Maes M. Long-COVID post-viral chronic fatigue and affective symptoms are associated with oxidative damage, lowered antioxidant defenses and inflammation: A proof of concept and mechanism study. *Mol Psychiatry.* 2023; 28(2): 564-578. doi: 10.1038/s41380-022-01836-9

29. Sharafati-Chaleshtori R, Shirzad H, Rafieian-Kopaei M, Soltani A. Melatonin and human mitochondrial diseases. *J Res Med Sci.* 2017; 22: 2. doi: 10.4103/1735-1995.199092

30. Camp OG, Bai D, Gonullu DC, Nayak N, Abu-Soud HM. Melatonin interferes with COVID-19 at several distinct ROS-related steps. *J Inorg Biochem.* 2021; 223: 111546. doi: 10.1016/j.jinorgbio.2021.111546

31. Polonikov A. Endogenous deficiency of glutathione as the most likely cause of serious manifestations and death in COVID-19 patients. *ACS Infect Dis.* 2020; 6: 1558-1562. doi: 10.1021/acsinfecdis.0c00288

32. Basi Z, Turkoglu V. *In vitro* effect of oxidized and reduced glutathione peptides on angiotensin converting enzyme purified from human plasma. *J Chromatogr B*. 2019; 1104: 190-195. doi: 10.1016/j.jchromb.2018.11.023

33. Esmaeili-Nadimi A, Imanparast F, Alizadeh S, Vatannejad A, Mohaghegh P, Seyedmehdi SM, et al. Total antioxidant capacity and total oxidant status and disease severity in a cohort study of COVID-19 patients. *Clin Lab*. 2023; 69(2). doi: 10.7754/Clin.Lab.2022.220416

34. Aykac K, Ozsurekci Y, Yayla BCC, Gurlevik SL, Oygar PD, Bolu NB, et al. Oxidant and antioxidant balance in patients with COVID-19. *Pediatr Pulmonol*. 2021; 56(9): 2803-2810. doi: 10.1002/ppul.25549

35. Çakırca G, Damar Çakırca T, Üstünel M, Torun A, Koyuncu İ. Thiol level and total oxidant/antioxidant status in patients with COVID-19 infection. *Ir J Med Sci*. 2022; 191(4): 1925-1930. doi: 10.1007/s11845-021-02743-8

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