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CHARACTERISTICS OF IRON-DEPENDENT PARAMETERS OF DONORS UNDER THE PRESENCE OF ANTI-SARS-CoV-2 IgG IN THE BLOOD

COVID-19 differs from other respiratory diseases in that it can cause an acute inflammatory reaction following widespread systemic complications in organisms. First, the inflammatory process causes an increase in the concentration of C-reactive protein (CRP), which could be a prognostic biomarker in patients with COVID-19. In addition, some clinical data were used to determine changes in the concentrations of ferritin and transferrin. Our study aimed to establish a relationship between the inflammatory process and iron-dependent parameters, as changes in concentration could lead to pathological status in the post-COVID-19 period. People sufferedfrom COVID-19 with different titers of anti-SARS-CoV-2 IgG in the blood participated in our experiment. It was established that the maximal concentration of CRP and ferritin was characterized for the donor group with a titer of anti-SARS-CoV-2 IgG 95 ± 5 Index (S/C) following the development of inflammatory anemia. Moreover, it was discovered that the group with a minimal titer of anti-SARS-CoV-2 IgG was characterized by the maximal concentration of transferrin, leading to the destruction of iron transport. Due to the acute inflammatory process and damage to the transport and storage of iron by transferrin and ferritin, the iron deficit could destroy the functioning of the muscle system. There was a change in the concentration of creatine kinase in the donor group with a titer of anti-SARS-CoV-2 IgG of 95 ± 5 Index (S/C). The study showed that infection with the SARS-CoV-2 virus in the body often leads to the development of acute inflammatory reactions, resulting in iron transport and storage processes, which cause pathological processes in the post-COVID-19 period.

Keywords: anti-SARS-CoV-2, ferritin, transferrin, C-reactive protein.

Introduction. Attention of scientists have been concentrating on the COVID-19 pandemic caused by the SARS-CoV-2 virus since 2020. According to clinical and statistical research, an abnormal inflammatory reaction to viral infection is observed in several patients, leading to multiple organ failure [1]. In general. developed on the background of the systemic inflammation influences the functional status of the organismand could be the reason for the pathology of the cardiovascular, nervous, digestive systemsand hemostasis in the post-COVID-19 period. Therefore, biological indicators of inflammatory reaction may be important for the evaluation of the COVID-19 stages.

The main inflammatory marker is a C-reactive protein (CRP) that influences on intensity of inflammation. CRP is a specific protein in the acute phase that synthesizes of hepatocytes and increases during infection and inflammation [2]. CRP activates the complement system that stimulates phagocytosis, cleaning organisms from pathogenic microorganisms [3]. The scientific research has reported that the concentration of CRP and the diameter of the maximum affection in the lungs increase with disease progression. The concentration of CRP was positively correlated with the effect of the lungs and disease stage. The concentration of CRP in the early stage of COVID-19 could demonstrate the status of the lungs and the degree of severity of the disease [4]. The concentration of CRP is known to be too high for bacterial and viral infections. However, research has shown that in patients with COVID-19 an increased the concentration of CRP is consistent with the above-mentioned clinical observations. In addition, an increase in the concentration of CRP was still observed in patients until disease progression [5].

Increased ofthe concentration of ferritin in the blood is possible in acute infectious diseases that could be linked to chronic inflammatory conditions [6]. Ferritin is a marker of the degree of severity and a prognostic factor for disease. According to the results of the clinical data, patients with COVID-19 and a decreased concentration of ferritin have an easy disease status. However, other patients with increased ferritin levels require intensive treatment and are more vulnerable to lethal consequences [7]. An increase in the concentration of ferritin is linked to the cytokine storm described in patients with COVID-19. Cytokine storms lead to the synthesis of more inflammatory cytokines, including IL-6, TNF-α, and IL-1β, which stimulate the secretion of ferritin by hepatocytes, Kupffer cells and macrophages. As a result, uncontrolled and dysfunctional immune responses caused by macrophage activation, hyperferritinemic syndrome and thrombotic storms lead to multiple organ dysfunction [8]. Moreover, changes in the concentration of ferritin are not only a consequence of unreasonable inflammation, but also play a pathogenic role in the development of inflammatory reactions due to their association with T-cell immunoglobulin and TIM-2 which stimulates the expression of more proinflammatory mediators [9].

Additianally, we hypothesized that transferrin could be a predictor of COVID-19. Virial infections cause a significant increase in the concentration of transferrin receptors in lungs. During the treatment of viral disease, blocking the transferrin receptor could decrease the pathogenesis of SARS-CoV-2, namely penetration into the cell. The precise mechanism of penetration SARS-CoV-2 into cells used for the transferrin receptor is unknown [10].

Therefore, our research aimed to study the changes in the concentration of the main biomarkers of the inflammatory process during the post-COVID-19 period – CRP and iron-containing parameters – ferritin and transferrin–to assess the interdependence of their functioning and impact on the general state of the body in the post-COVID-19 period.

The target of our work wasto investigate potential changes in the concentration of iron-containing parameters such as ferritin, transferrin, and the biomarker of acute inflammatory reaction – CRP in experimental donor groups.

Materials and methods. People sufferedwith COVID-19 and agreed to be donors of blood plasma participated in our experiment. Blood plasma was collected from donors to 3–4 months after COVID-19. We sent the blood plasma of

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donors with determined titers of anti-SARS-CoV-2 IgG for further scientific research.

Anti-SARS-CoV-2 IgG titers in the blood plasma were determined by chemiluminescent microparticle immunoassay technology using the Abbott SARS-CoV-2 IgG assay (Abbott Diagnostics, Abbott Park, Illinois, United States). All donors were selected in groups based on the anti-SARS-CoV-2 IgG titer. As result, we have had such donor groups with titers of anti-SARS-CoV-2 IgG: 5 ± 3 (n = 20); 55 ± 5 (n = 20); 65 ± 5 (n = 20); 75 ± 5 (n = 20); 85 ± 5 (n = 20); 95 ± 5 (n = 20); 125 ± 5 (n = 20); 175 ± 5 (n = 20) Index (S/C). Thus, donors without anti-SARS-CoV-2 IgG were chosen as the control group.

All donors voluntarily agreed to participate in the clinical experiment and provided written, informed consent.

The concentration of ferritin was determined with a sandwich-type immunochemiluminescent assay using a ferritin test kit for CLIA Maglumi (Snibe Co., Ltd., China). All manipulations were performed in accordance with the manufacturer's instructions. The measured results are shown as relative luminescence units (RLU), which are proportional to the ferritin concentration in the samples [11].

The concentration of C-reactive protein was measured with a sandwich-type immunochemiluminescent assay using a CRP Test Kit for CLIA Maglumi (Snibe Co., Ltd., China). All manipulations were carried out according to the manufacturer's instructions. The measured results are shown as relative light units (RLU) and are proportional to the concentration of C-reactive protein in the samples [12].

The concentrations of transferrin and creatine kinase were measured using standard test kits for the biochemical analyzer Humalyser 3000 [13].

Statistical processing of the obtained results was performed with various statistical methods using the computer programStatistic Statistics 10. The arithmetic mean (M) and mean squared error (MSE) indicators were calculated. The hypothesis of normal distribution was checked using the Shapiro-Wilk and Kolmogorov-Smirnov tests. All donor groups showed a non-normal distribution. Therefore, the differences between the samples were determined using Kruskal-Wallis-Test. A statistically significant test result ($p < 0,05$) was considered reliable.

Results and discussed. We studied the potential changes in the concentration of iron-containing parameters and their relationship with inflammatory parameters in the post-COVID-19 period in the experimental and control donor groups. This paper was demonstrated the donor groups with maximal and minimal concentrations of the research parameters.

The concentration of CRP was analyzed in the plasma of all donors. Based on the obtained results, we determined that the maximal concentration of CRP was characterized for donors withtiter of anti-SARS-CoV-2 IgG 95 ± 5 Index (S/C) compared to the control group. Then, the minimal concentration of CRP was established in donors with titer of anti-SARS-CoV-2 IgG 75 ± 5 Index (S/C) among the experimental donor groups **(Fig. 1)**.

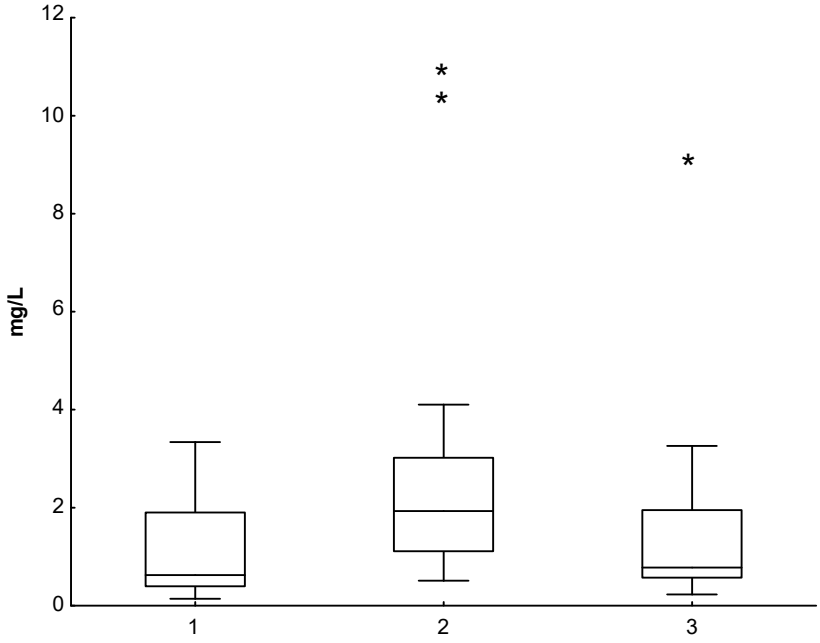

Figure 1. The concentration of C-reactive protein in donor groups with such titers of anti-SARS-CoV-2 IgG: 1 – 0 Index (S/C); 2 – 95 ± 5 Index (S/C); 3 – 75 ± 5 Index (S/C), p < 0,05

We evaluated the changes in iron-dependent parameters, such as transferrin and ferritin, in the plasma of all donor groups. It was established that the maximal concentration of ferritin had donors with titer of anti-SARS-CoV-2 IgG 95 ± 5 Index (S/C) then minimal – 175 ± 5 Index (S/C) compared to control group and among all experimental groups **(Fig. 2)**.

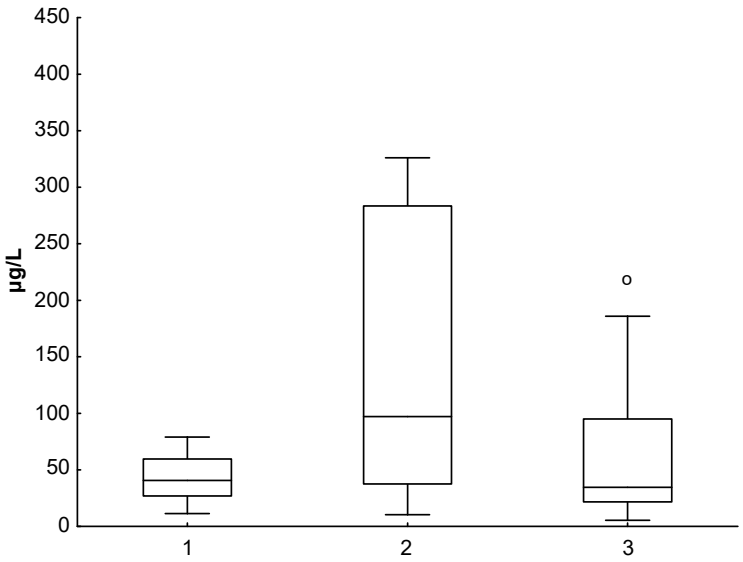

Figure 2. The concentration of ferritin in donor groups with such titers of anti-SARS-CoV-2 IgG: 1 – 0 Index (S/C); 2 – 95 ± 5 Index (S/C); 3 – 175 ± 5 Index (S/C), p < 0,05

Moreover, in this experiment, we investigated the potential changes in the concentration of the main transporter of iron – transferrin in the plasma of all groups. It was determined that the maximal concentration of transferrin was found in the donor group with titer of anti-SARS-CoV-2 IgG 5 ± 3 Index (S/C) while the minimal – 125 ± 5 Index (S/C) compared to the control donor group **(Fig. 3)**.

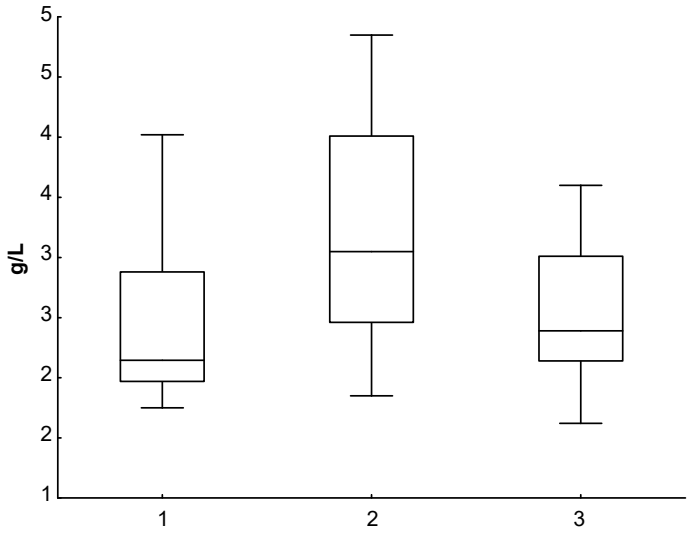

Figure 3. The concentration of transferrin in donor groups with such titers of anti-SARS-CoV-2 IgG: 1 – 0 Index (S/C); 2 – 5 ± 3 Index (S/C); 3 – 125 ± 5 Index (S/C), p < 0,05

The literature data is known that an increase of content of the intracellular iron in the body leads to an increase of concentration of ferritin because of the iron keeps to ferritin form and is removed from the cell at future [14]. The infection process leads to the utilization of ferritin in tissues or the liquidation of iron-containing heme and hemoglobin from the bloodstream to save ferritin in macrophages. The increased iron absorption and isolation by macrophages provides the realization of such functions: to prevent the penetration of iron into pathogenic organisms and to protect the patient against the toxic influence of increased concentrations of iron, heme, and hemoglobin, which could exude during the destruction of tissues during infection and inflammation [15]. In addition, an increase in the concentration of ferritin is connected not only to the inflammatory process, but could also be an indirect indicator of cell damage. Ferroptosis caused by the excessive accumulation of ferritin has been described in acute respiratory distress syndrome, which occurs in the clinical practice of COVID-19 [16].

In the experiment, the maximal concentration of CRP and ferritin was characterized for the donor group with titers of anti-SARS-CoV-2 IgG 95 ± 5 Index (S/C) among all experimental groups. CRP is one of the key biomarkers of inflammatory processes in organisms that primarily activate immune cells, such as macrophages [17]. Following to described above, the macrophages are considered to the main source of synthesis of ferritin. Ferritin accumulates during the acute inflammatory

reaction and captures and stores more iron. These processes lead to the development of inflammatory anemia as a protective mechanism [18], including a decrease in the concentration of circulating iron to prevent the penetration and spread of SARS-CoV-2 in the body.

Another study reported that iron deficiency could be the reason for the bioenergetic shortage in muscles following the development of muscle atrophy [19]. In patients with COVID-19, an increase in the concentration of total creatine kinase and creatine kinase-MB results in the destruction of muscle tissues [20]. According to the experimental results, an increase in the concentration of ferritin in some donor groups during the inflammatory reaction could be due to the destruction of the physiological and functional state of muscle tissues. **Fig. 4** shows that an increase in total creatine kinase is characterized for the donor group with a titer of anti-SARS-CoV-2 IgG 95 ± 5 Index (S/C) compared to the control donor group. This means that the risk of development of muscle atrophy increases in this group, which could be connected with an increase in ferritin concentration causing an iron deficit in the bloodstream because iron is necessary for the function of muscle tissues.

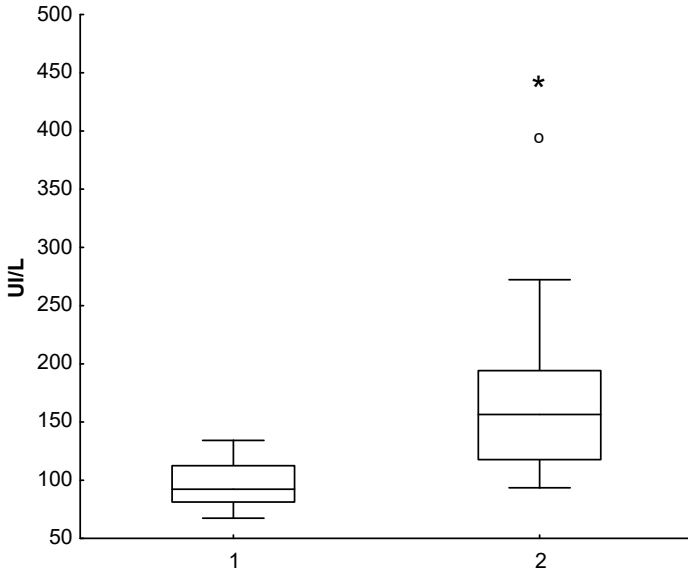

Figure 4. The concentration of creatinekinase in donor groups with such titers of anti-SARS-CoV-2 IgG: 1 – 0 Index (S/C); 2 – 95 ± 5 Index (S/C), p < 0,05

Currently, the literature does not report a decrease in ferritin concentration during COVID-19. Our research showed that the donor group with an titer of anti-SARS-CoV-2 IgG 175 ± 5 Index (S/C) had the lowest ferritin concentration among all experimental groups. Moreover, 36 % of the donors in this group had a lower ferritin concentration than the control donor group. In general, an abnormal decrease in ferritin concentration was observed in donors with a maximal titer of anti-SARS-CoV-2 IgG of 175 ± 5 Index (S/C). This could be linked to the potential influence of IgG on iron metabolism in organisms or the depletion of iron reserves due to the widespread use of SARS-CoV-2 and acute inflammatory reactions.

Transferrin plays an equally important role in iron transport during erythropoiesis. It has been reported that SARS-CoV-2 can directly influence transferrin in the processes of iron transport, leading to non-affective erythropoiesis and iron deficiency anemia [21, 22]. An increase in the transferrin concentration and changes in the transferrin/antithrombin III (ATIII) ratio could damage the regulation of coagulation, causing the development of coagulopathy linked to COVID-19 [23, 24]. Our research established that the donor group with a titer of anti-SARS-CoV-2 IgG 125 ± 5 index (S/C) had a decrease in the concentration of transferrin. It is assumed that the penetration and accumulation of SARS-CoV-2 in organisms causes a hyperinflammatory reaction that leads to a decrease in the concentration of transferrin which is the main iron carrier. We emphasize that the maximal concentration of transferrin was characterized in the donor group with a minimal titer of anti-SARS-CoV-2 IgG 5 ± 3 index (S/C). As a result, such processes could influence the development of coagulation by blocking of the inactivating effect of ATIII on coagulation proteases by binding to ATIII.

Infection with SARS-CoV-2 induces immunoinflammatory reactions in an organism, leading to an increase in the concentrations of ferritin and transferrin. To protect against the negative influence of the pathogen, iron chelated by ferritin in its reserve form is a risk factor for the development of iron deficiency diseases. Notably, the deficit of functional active iron causes the destruction of the working status of muscle tissues according to our research – a decrease in the concentration of creatinekinase in the experimental group compared to that in the control group. In addition, an abnormal concentration of transferrin is not only an imbalance in iron metabolism but also a negative factor influencing hemostasis.

Conclusions. The experiment was obtained the changes of concentration of the inflammatory biomarker C-reactive protein, and iron-dependent parameters such as ferritin and transferrin in the blood plasma. We suggest that the acute inflammatory reaction influences the functional status of ferritin and transferrin, leading to the development of systemic complications in the post-COVID-19 period. The research results could be beneficial for the improvement and search for new therapeutic methods for COVID-19, and emphasize the need for careful laboratory diagnosis of patients to avoid possible complications.

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ХАРАКТЕРИСТИКА ЗАЛІЗОЗАЛЕЖНИХ ПАРАМЕТРІВ ДОНОРІВ ЗА НАЯВНОСТІ АНТИ-SARS-CoV-2 IgG У КРОВОТОЦІ

COVID-19 відрізняється від інших респіраторних захворювань тим, що викликає гостру запальну реакцію, унаслідок якої може поширюватися системне ураження в організмі. Запальний процес супроводжується передусім зростанням концентрації С-реактивного білка (СРБ), що може бути одним із прогностичних біомаркерів перебігу COVID-19 у пацієнтів. Крім цього, на тлі таких подій у деяких клінічних спостереженнях зафіксовано зміни концентрацій феритину і трансферину. У нашому дослідженні увага зосереджена на встановленні взаємозв'язку між запальними процесами та залізозалежними параметрами, зміни концентрацій яких можуть призвести до патологічних станів у пост-COVID-19 періоді. В експерименті брали участь люди, які перехворіли на COVID-19 та у кровотоці яких наявні різні титри анти-SARS-CoV-2 IgG. Установлено, що для групи донорів із титром анти-SARS-CoV-2 IgG 95 ± 5 Index (S/C) характерні максимальні концентрації С-реактивного білка та феритину, унаслідок чого може розвиватися запальна анемія. Також виявлено, що у групи з мінімальним титром анти-SARS-CoV-2 IgG спостерігається максимальна концентрація трансферину, що може свідчити про порушення процесів транспорту заліза. Крім цього, нестача заліза внаслідок гострого запального процесу, який супроводжується пошкодженням транспорту та запасання заліза трансферином і феритином, відповідно, може порушувати роботу м'язової системи, що підтверджується зміною концентрації креатинкінази у групи донорів із титром анти-SARS-CoV-2 IgG 95 ± 5 Index (S/C). Проведене дослідження свідчить, що зараження вірусом SARS-CoV-2 організму часто призводить до розвитку гострих запальних реакцій, унаслідок яких можуть страждати процеси транспорту й запасання заліза, які викликатимуть патологічні процеси у пост-COVID-19 періоді.

Ключові слова: анти-SARS-CoV-2 IgG, феритин, трансферин, С-реактивний білок.