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AMYLASE CONCENTRATION IN DONOR GROUPS DEPENDING ON TITERS OF ANTI-SARS-CoV-2 IgG IN BLOOD PLASMA

COVID-19 is a disease of the respiratory system; however, some patients experience multiorgan complications, including those of the digestive system. Many studies have focused on liver, bile duct and stomach dysfunctions during the SARS-CoV-2 infection. Currently, it is known that COVID-19 leads to changes in amylase concentration in the blood, which may be a prognostic factor for pancreatic damage; however, information regarding these clinical cases is limited. Our study aimed to determine the potential changes in total amylase, pancreatic amylase and C-reactive protein (CRP) levels in the blood of the donor groups with different titers of anti-SARS-CoV-2 IgG. Donor groups with titers of anti-SARS-CoV-2 IgG 10 ± 3 and 95 ± 5 Index (S/C) were characterized by an increase in total and pancreatic amylase concentrations compared to the donor group with titer of anti-SARS-CoV-2 IgG 0 Index (S/C). We assumed that multiorgan dysfunction developed through the acute inflammatory reactions caused by the SARS-CoV-2 infection. In particular, this may lead to pancreatic complications. To confirm this, we analyzed the CRP concentration in the donor group with the changes in amylase concentration compared to the donor group with titer 0 Index (S/C). The results obtained may be useful in medical practice for diagnosing complications in the post-COVID-19 period and for preventing the development of pancreatic dysfunction during the development of an algorithm for the treatment of COVID-19.

Keywords: amylase, C-reactive protein, anti-SARS-CoV-2 IgG.

Introduction. A wide range of viral and bacterial infections, including acute respiratory diseases, may lead to digestive disorders. The literature reports reasons for gastrointestinal tract dysfunction during infection processes in the body, such as co-infections between respiratory pathogens and enteroviruses, side effects of drug treatment and direct/indirect influence of respiratory pathogens [1, 2, 3]. COVID-19 is one of type a respiratory disease that leads to multiorgan dysfunction in patients [4]. Although the main target of SARS-CoV-2 is the respiratory pathways, there are clinical cases of patients with symptoms such as nausea, vomiting, diarrhea, and stomach pain that may indicate digestive disorders [5, 6].

Currently, there are some investigations regarding the development of acute pancreatitis in patients with SARS-CoV-2 infection [7, 8]. Moreover, the effect of COVID-19 on the pancreas remains unknown [9]. The pancreas produces amylase; therefore, the increase in amylase concentration may be caused by organ inflammation. In addition, amylase is synthesized by the salivary glands [10]. As a result, changes in amylase concentration may be caused by salivary gland disorders [11]. The primary investigation reports that 17 % of patients were characterized by pancreas complications. Moreover, some studies have demonstrated that 13.5 % of patients in the intensive care unit have higher amylase concentrations [12, 13]. Scientists have concluded that SARS-CoV-2 causes mild pancreatic dysfunction. It is assumed that an increase in amylase concentration may also be caused by a nonpancreatic etiology. Patients with COVID-19 sometimes have symptoms of acidosis and diarrhea, which lead to an increase in amylase concentration [14, 15]. Diarrhea and intestinal inflammation increase the absorption of amylase and lipase into the intestinal lumen with further absorption into the blood. As the kidneys are the main site for the removal of excess amylase, hyperamylasemia may be detected because of kidney dysfunction [16, 17].

In summary, SARS-CoV-2 infection negatively influences the pancreas, as confirmed by the diagnostic test results of changes in the amylase concentration. In addition, there are different predictions regarding the mechanisms underlying the influence of SARS-CoV-2 on the physiological and functional states of the pancreas and related organs that participate in amylase production. Currently, there is insufficient information regarding the relationship between post-COVID-19 period and changes in blood amylase concentration.

The aim of our research was to investigate the potential changes in amylase concentration in the blood plasma of recovered from COVID-19 donors with different titers of anti-SARS-CoV-2 IgG.

Materials and methods. People who had suffered from COVID-19 agreed to be donors of blood plasma for biotechnological purposes at "BIOPHARMA-PLASMA" (Kyiv, Ukraine). The donors ranged in age from 25 to 45 years old. Blood plasma was collected from donors who had recovered from COVID-19 3-6 months ago. Donors were checked by screening tests before blood plasma was used to produce the targeted biotechnological drugs. Individuals with severe cardiovascular and cerebrovascular diseases, vitamin K deficiency, traumatic coagulation disorders, anticoagulant therapy or other disorders that can affect the hemostasis system were excluded from the study. The blood plasma of donors was used to determine anti-SARS-CoV-2 IgG titers for scientific research.

The titers of anti-SARS-CoV-2 IgG in blood plasma were determined using a chemiluminescent microparticle immunoassay (Abbott Laboratories, USA) according to the manufacturer's instructions. All donors were selected based on anti-SARS-CoV-2 IgG titers. We had donor groups with anti-SARS-CoV-2 IgG titers: 0 (n=20), 10 ± 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) and 175 ± 5 (n=20) Index (S/C). All donors voluntarily agreed to participate in the study and provided written informed consent.

Total and pancreatic amylase concentrations were measured using a biochemistryanalyzer Humalyser 3000 with standard test kits [18]. The C-reactive protein concentration was determined by immunochemiluminescence analysis of the "sandwich" type according to the manufacturer's instructions [19].

The statistical processing of the obtained results was carried out using the STATISTICA 12 program (StatSoft Inc.). Testing of the hypothesis of a normal distribution of choices was done by Shapiro-Wilk and Kolmogorov-Smirnov tests. It was established that the experimental

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groups of donors do not obey the normal distribution; therefore, the differences between the selections were determined using the Kruskal–Wallis test.

Results and discussion. This study detected changes in total and pancreatic amylase in the blood plasma of donor groups. The most significant differences in groups with titers of anti-SARS-CoV-2 IgG ≥ 10 ± 3 Index (S/C) compared to the donor group with titer of anti-SARS-CoV-2 IgG 0 Index (S/C) are presented below.

According to the obtained results, an increase in total amylase concentration in blood plasma was detected in donor groups with titers of anti-SARS-CoV-2 IgG 10 ± 3 and 95 ± 5 Index (S/C), Figure 1. Among other donor groups with titers of anti-SARS-CoV-2 IgG 55 ± 5, 65 ± 5, 75 ± 5, 85 ± 5, 125 ± 5, 175 ± 5 Index (S/C) were not determined to be statistically significant compared to the donor with titer of anti-SARS-CoV-2 IgG 0 Index (S/C).

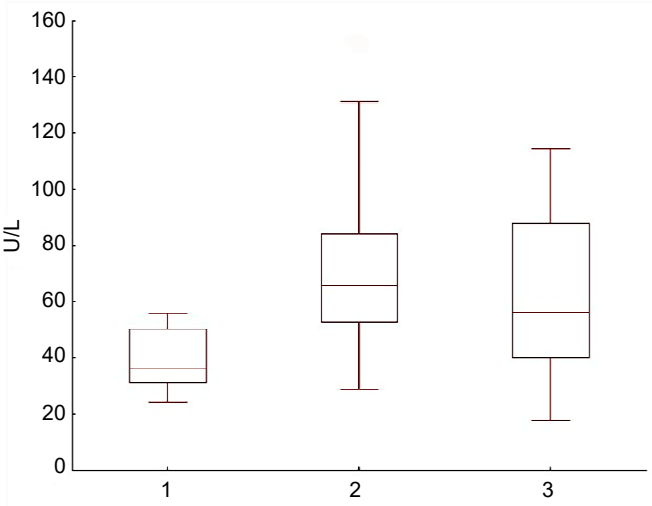

Fig. 1. Total amylase concentration in blood plasma of donor groups with different titers of anti-SARS-CoV-2 IgG: 1 – 0 Index (S/C); 2 – 10 ± 3 Index (S/C); 3 – 95 ± 5 Index (S/C)

$p < 0.05$ in donor groups with titers of anti-SARS-CoV-2 IgG 10 ± 3 Index (S/C); and 95 ± 5 Index (S/C) compared to donor group with titer of anti-SARS-CoV-2 IgG 0 Index (S/C).

The next step was to establish changes in pancreatic amylase concentration in the donor groups. The increase and maximum concentration of pancreatic amylase was found in the donor group with titer of anti-SARS-CoV-2 IgG 10 ± 3 Index (S/C) among donor groups with titers of anti-SARS-CoV-2 IgG ≥ 10 ± 3 Index (S/C). In addition, a statistically significant difference in pancreatic amylase concentration was determined in the donor group with titer of anti-SARS-CoV-2 IgG 95 ± 5 Index (S/C) compared to reference point – group with titer of anti-SARS-CoV-2 IgG 0 Index (S/C), Figure 2. Other donor groups with titers of anti-SARS-CoV-2 IgG 55 ± 5, 65 ± 5, 75 ± 5, 85 ± 5, 125 ± 5, 175 ± 5 Index (S/C) were not significantly different from the donor group with titer of anti-SARS-CoV-2 IgG 0 Index (S/C).

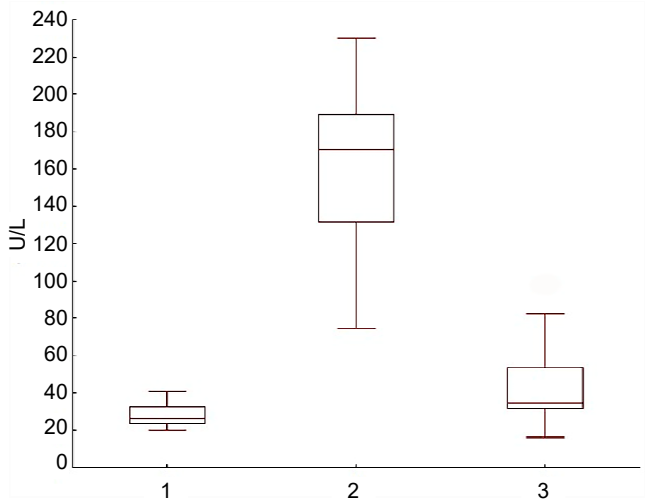

Fig. 2. Pancreatic amylase concentration in blood plasma of donor groups with different titers of anti-SARS-CoV-2 IgG: 1 – 0 Index (S/C); 2 – 10 ± 3 Index (S/C); 3 – 95 ± 5 Index (S/C)

$p < 0.05$ in donor groups with titers of anti-SARS-CoV-2 IgG 10 ± 3 Index (S/C); and 95 ± 5 Index (S/C) compared to donor group with titer of anti-SARS-CoV-2 IgG 0 Index (S/C).

Previous research has demonstrated that patients with COVID-19 belong to the high-risk group of pancreatic disorders according to laboratory analysis of the increase in total amylase concentration in the blood [20, 21]. Our results showed that donor groups with titers of anti-SARS-CoV-2 IgG 10 ± 3 and 95 ± 5 Index (S/C) were characterized by the increase in total amylase concentration compared to the donor group with titer of anti-SARS-CoV-2 IgG 0 Index (S/C). As a result, the increase in pancreatic amylase concentration indicates the probability of pancreatic damage during post-COVID-19 period.

There may be several reasons for the occurrence of pancreatic disorders. We predict that the destruction of pancreatic function may be caused by acute immune responses related to SARS-CoV-2 infection [22, 23]. We analyzed the changes in CRP concentration as the main biomarker of inflammation in donor groups with titers of anti-SARS-CoV-2 IgG 10 ± 3 and 95 ± 5 Index (S/C). According to the results (Table 1), there was an increase in CRP concentration in the experimental groups compared to the donor group with anti-SARS-CoV-2 IgG titer 0 Index (S/C).

Table 1. **CRP concentration in blood plasma of donor groups with different titers of anti-SARS-CoV-2 IgG**

Titer of anti-SARS-CoV-2 IgG, Index (S/C)	CRP concentration in blood plasma [Q1-Q3], mg/mL
0	0.480 [0.350-0.740]
10 ± 3	1.110 [0.580-2.180], $p < 0.05$
95 ± 5	1.910 [1.105-2.895], $p < 0.05$

Moreover, the epithelial cells lining the ducts of the salivary glands were the primary targets of SARS-CoV in experimental model of infected SARS-CoV rats [24]. We assume that SARS-CoV-2 infection may cause similar reactions that lead to an increase in total amylase concentration along with an increase in pancreatic amylase in the blood plasma. Any physiological changes, including increased vascular permeability, impaired lymph outflow and abnormal clearance, can lead to hyperamylasemia in the blood. Damage to the hepatic excretion of amylase may be an important cause of hyperamylasemia [25].

Thus, our research demonstrates that an increase in total and pancreatic amylase levels linked to pancreatic dysfunction may cause systemic inflammation or an indirect influence of SARS-CoV-2 on organ cells. We underline that among donor groups depending on titers of anti-SARS-CoV-2 the most significant changes of amylase concentration were detected only in donor groups with titers of anti-SARS-CoV-2 IgG 10 ± 3 and 95 ± 5 Index (S/C). Also, we assume that these processes may be caused by IgG, which circulates in blood plasma, and described that these donor groups experience the greatest dysfunction in amylase concentration.

Conclusion. This study describes the increase in amylase levels, which may indicate pancreatic dysfunction in the post-COVID-19 period. Assumption has been confirmed to increase pancreatic amylase concentration in defined donor groups. These processes may be linked to inflammatory reactions in organisms, because there are changes in CRP concentration as a predictor of acute immune status in groups. Therefore, this research may be appropriate for the development of therapeutic therapies for COVID-19 and for the prevention of pathological conditions in the post-COVID-19 period.

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ХАРАКТЕРИСТИКА КОНЦЕНТРАЦІЙ АМІЛАЗИ У ГРУП ДОНОРІВ ЗАЛЕЖНО ВІД ТИТРІВ АНТИ-SARS-CoV-2 IgG У ПЛАЗМІ КРОВІ

Насамперед COVID-19 – захворювання органів дихальної системи, однак більшість пацієнтів страждає від позалегеневих ускладнень, які, у тому числі, впливають на роботу шлунково-кишкового тракту. Чимало досліджень зосереджені на проблемах функціонування печінки, жовчних проток і розладах шлунку під час інфікування SARS-CoV-2. Наразі відомо, що захворювання на COVID-19 викликає зміни концентрацій амілази у крові, що може бути прогностичним фактором порушень роботи підшлункової залози, однак існує недостатньо інформації про такі клінічні випадки. Наше дослідження було зосереджене на діагностиці потенційних змін концентрацій загальної амілази, панкреатичної амілази та С-реактивного білка у плазмі крові донорів, які перехворіли на COVID-19 і мають різні титри анти-SARS-CoV-2 IgG у кровотоці. Установлено, що у групах донорів із титрами анти-SARS-CoV-2 IgG 10 ± 3 та 95 ± 5 Index (S/C) зростають концентрації загальної і панкреатичної амілази у крові порівняно із групою донорів, у кровотоці яких відсутні анти-SARS-CoV-2 IgG. Ми припускаємо, що такі зміни виникають внаслідок гострої запальної реакції, викликаної інфікуванням SARS-CoV-2, і можуть спричинити ускладнення у роботі підшлункової залози. Для підтвердження було проаналізовано концентрації С-реактивного білка (СРБ) у плазмі крові груп донорів із найбільшими відхиленнями концентрацій амілази. Виявлено, що у групах донорів із титрами анти-SARS-CoV-2 IgG 10 ± 3 та 95 ± 5 Index (S/C) зростають концентрації СРБ порівняно із групою донорів без анти-SARS-CoV-2 IgG у кровотоці. Проведене дослідження може бути корисним у лікувальній практиці для діагностики ускладнень у пост-COVID-19 періоді та для запобігання розвитку порушень роботи підшлункової залози під час розробки алгоритму терапії захворювання COVID-19 у пацієнтів.

Ключові слова: амілаза, С-реактивний білок, анти-SARS-CoV-2.