Metabolic abnormalities in patients with non-alcoholic fatty liver disease with immune response to SARS-CoV-2

Abstract. Background. It is known that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can directly infect hepatocytes. At the same time, overweight and obesity are surrogate markers of the adverse effects of coronavirus disease 2019 (COVID-19). The purpose of the study: to evaluate changes in lipid and carbohydrate metabolism and their indices in the serum of patients with non-alcoholic fatty liver disease (NAFLD) with an immune response to SARS-CoV-2. Materials and methods. We studied 37 patients with NAFLD who had IgG to SARS-CoV-2. All patients were divided into two groups: group I consisted of 19 participants who were PCR-negative for SARS-CoV-2, group II included 18 patients who had COVID-19, as confirmed by PCR testing. The content of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), glucose, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol (VLDL-C), atherogenicity coefficient, insulin resistance indices (HOMA-IR), TG to glucose (TG/Gly) and TG to HDL-C (TG/HDL-C) ratio were assessed in all patients. Results. Among patients with NAFLD with an immune response to SARS-CoV-2, dyslipidemia manifested by a probable increase in the median TG content in groups I and II by 2.5 and 3.4 times (p = 0.0001), respectively; TC — by 1.2 times (p = 0.0425) in both groups, with a tendency to increase; VLDL-C — by 1.8 (p = 0.010) and 2.5 times (p = 0.0022), respectively, and a decrease in the median content of HDL-C by 1.7 (p = 0.0016) and 1.9 times (p = 0.0008), respectively, in blood serum. The identified changes led to a statistically significant increase in the median atherogenicity coefficient in groups I and II — by 2.2 (p = 0.0036) and 2.5 times (p = 0.007). An increase in the HOMA-IR did not have a statistically significant difference between the groups: in group I, this index increased by 3.1 times (p < 0.05) compared with the controls, in group II — by 3 times (p < 0.05). In addition, patients in both groups had a statistically significant increase in TG/Gly and TG/HDL-C ratio compared to controls. However, the detected changes were more pronounced in group II, where TG/Gly and TG/HDL-C levels were 1.5 (p = 0.038) and 1.9 times (p = 0.035), respectively, higher than in group I. Conclusions. Patients with an immune response to SARS-CoV-2 with NAFLD have disorders of lipid and carbohydrate metabolism. At the same time, the signs of dyslipidemia were more pronounced in participants with a history of SARS-CoV-2. TG/Gly and TG/HDL-C ratio should be included in the diagnostic algorithm for assessing insulin resistance in patients with COVID-19 who are overweight or obese. Keywords: non-alcoholic fatty liver disease; SARS-CoV-2; lipid metabolism; carbohydrate metabolism

Introduction
Since 2019, the outbreak of coronavirus disease 2019 (COVID-19), caused by the novel coronavirus CoV-2 (SARS-CoV-2) in severe acute respiratory syndrome (SARS), has led to a major global health and economic crisis in a few months. As of April 2022, more than 507 million confirmed cases were reported worldwide, with nearly 6.2 million deaths in 189 countries [1]. Liver damage in COVID-19 may correlate with the overall severity of the disease and serve as a prognostic factor for the development of acute respiratory distress syndrome [2]. In addition, some studies have shown that the presence of pre-existing liver disease in patients with COVID-19 contributes to poor clinical outcomes and should be taken seriously during treatment, particularly in liver transplant patients [3] with cirrhosis, acute liver injury [4] and COVID-19, due to an altered immune status...
and susceptibility to disease. Therefore, further research in patients with liver disease requires a better understanding of the pathogenesis and optimal treatment of COVID-19. Severe liver damage was observed in 58–78 % of patients with severe clinical manifestations. Thus, liver damage is a surrogate marker of the adverse effects of COVID-19 [4, 5]. In addition, existing liver diseases such as chronic viral hepatitis, steatohepatitis, liver cirrhosis, and hepatoacellular carcinoma are independent risk factors for the severity and mortality of the disease [6, 7]. However, most studies have suggested that angiotensin-converting enzyme 2 (ACE2) may be low or absent in hepatocytes, and its expression was greater in biliary cells than in hepatocytes. Through ultrastructural examination, a recent study has shown that typical coronavirus particles can be found in the cytoplasm of hepatocytes from COVID-19 patients, indicating that SARS-CoV-2 is able to directly infect hepatocytes in some cases [8]. Thus, whether liver damage is caused by severe viral impact, immune-mediated liver damage due to severe inflammatory response/systemic inflammatory response syndrome, hypoxic changes caused by respiratory failure, vascular changes due to coagulopathy, endotheliitis, or cardiac congestion with right-sided heart failure, drug-induced liver damage, and exacerbation of underlying liver disease remains unknown (Fig. 1) [5, 9].

Non-alcoholic fatty liver disease (NAFLD) in patients with SARS-CoV-2 infection is associated with impaired lipid metabolism, namely, increased serum fatty acid concentrations, increased fatty acid synthesis in the liver, and abnormal β-oxidation. 60 % of triglycerides (TG) in the liver are formed from absorbed unesterified fatty acids. In NAFLD, lipid intake exceeds lipid utilization, as there is increased fatty acid absorption, increased TG lipolysis in adipose tissue, and increased de novo lipogenesis in the liver. Increased de novo lipogenesis is associated with upregulation of fatty acid synthase, elongase 6, and sterol-CoA desaturase, and these 3 enzymes are regulated by important transcription factors such as sterol regulatory element-binding protein (SREBP), liver X receptor, and carbohydrate response element-binding protein [9, 10]. Hypolipidemia is a rare condition, and it can be caused by a genetic change or secondary factors such as a viral infection. For example, in patients infected with SARS-CoV-2, liver function is damaged and thereby high- (HDL) and low-density lipoprotein (LDL) biosynthesis is reduced by decreasing the outflow and transport of cholesterol, but the virus can also change vascular permeability, causing leakage of cholesterol molecules into tissues such as alveolar spaces, with the formation of exudate.

Recently, the triglyceride-glucose (TG/Gly) and the TG/HDL-C ratio have been actively used as indicators for assessing insulin resistance. TG/Gly is the ratio of fasting blood glucose to TG [10]. A positive correlation between the TG/Gly index and insulin resistance has been shown. Compared with the homeostasis model assessment of insulin resistance (HOMA-IR) and the hyperinsulinemic-euglycemic clamp, TG/Gly is a new and reliable alternative index for assessing IR. In addition, an increase in TG/HbA1c is associated with an increased risk of developing type 2 diabetes, and this index is positively correlated with coronary artery calcification and is an effective indicator for predicting the progression of cardiovascular disease [11]. A study of 9,764 middle-aged and elderly Chinese adults (mean age 56 years) showed that compared to other blood lipid markers, TG/HDL-C may be a better indicator for assessing insulin resistance and diabetes. TG/HDL-C was used as a surrogate indicator of insulin resistance in people with pulmonary hypertension without a history of diabetes and TG/HDL-C > 3.0 was an indicator of IR [10].

IR, oxidative stress, and inflammatory processes play an important role in the development of NAFLD/non-alcoholic steatohepatitis (NASH). It is assumed that increased production of proinflammatory cytokines plays a special role in the pathogenesis of liver IR. The involvement of the liver in immune reactions and inflammation is an important aspect of the pathophysiology of NAFLD. Tumor necrosis factor α (TNF-α) has been shown to enhance IR, activate lipogenesis in the liver and increase serum TG levels, i.e., contribute to

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**Figure 1 — Pathomechanisms of liver damage in SARS-CoV-2 infection**
the main pathogenetic mechanisms of NAFLD/NASH [12, 13]. Recent studies have begun to shed light on the relationship between liver steatosis, which until now was considered a purely “metabolic disease,” and IR, which is associated with obesity and type 2 diabetes mellitus [7]. Patients with both NAFLD and metabolic syndrome have similar risk factors: obesity, IR, hypertension, hyperglycemia, and dyslipidemia [14]. Because of the crucial role of TG/Gly and TG/HDL-C in insulin resistance, this study aimed to use TG/Gly and TG/HDL-C to assess the risk of developing and progressing insulin resistance in patients with NAFLD.

The purpose of the study is to evaluate changes in lipid and carbohydrate metabolism and their indices in the serum of patients with non-alcoholic fatty liver disease with an immune response to SARS-CoV-2.

Materials and methods

We examined 37 patients with NAFLD who had detected IgG to SARS-CoV-2 and were treated at the Department of Liver and Pancreas Diseases of the State Institution “Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine”. There were 31 (83.8 %) men and 6 (16.2 %) women, with a median age of 43 (36; 54) years. Group I consisted of 19 patients who were PCR-negative for SARS-CoV-2. Group II included 18 patients with COVID-19, as confirmed by the polymerase chain reaction. Patients in both groups were vaccinated against coronavirus. The control group consisted of 20 practically healthy individuals. The study was performed in compliance with the basic bioethical provisions of the Council of Europe Convention on Human Rights and Biomedicine (April 4, 1997), the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. The study was approved by the Biomedical Ethics Committee of the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine (extract from protocol No. 2 dated April 19, 2021).

Lipid and carbohydrate metabolism was assessed using venous blood taken in the morning on an empty stomach. Serum levels of total cholesterol (TC), TG, HDL-C, and glucose were determined using Cormay reagent kits (Poland) on a Stat Fax 4500 biochemical analyzer (Awareness Technology, USA). Low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and atherogenicity coefficient (AC) were calculated according to the formula of W.T. Friedewald and co-authors. TG/Gly and TG/HDL-C ratios were calculated by dividing the serum concentration of TG by glucose and TG by HDL-C measured in mmol/l.

The level of insulin in blood serum was determined by enzyme-linked immunosorbent assay (ELISA) using test systems of the Chema company (Ukraine). HOMA-IR was calculated according to the formula: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (μU/ml) / 22.5. ELISA was performed using Stat Fax 303 Plus analyzer (USA), on which optical density was measured at a wavelength of 450 nm.

Statistical processing of the results was performed using the Statistica 10.0 software package. The compliance of the data distribution with the law of normal distribution was checked using the Shapiro-Wilk method. The median (Me), lower (Q1) and upper (Q3) quartiles were used to describe the data. The comparison of indicators between groups was carried out using a nonparametric method (Mann-Whitney U test). Statistical significance was assessed at a level not lower than 95.0 % (p < 0.05).

Results

The analysis of the data showed statistically significant differences in most lipid metabolism parameters in patients of both groups compared to the controls. However, the most profound lipid metabolism disorders were characteristic of patients of group II (Table 1).

Researchers observed the presence of hypolipidemia associated with SARS, which was manifested by low levels of TG, TC, HDL, and LDL in the blood serum (active phase of the disease) [15]. Alterations in fatty acid metabolism in patients with NAFLD (data presented above) may also contribute to the development of dyslipidemia reported in COVID-19 [16]. In our study, dyslipidemia manifested by a significant increase in the median TC content in groups I and II by 2.5 and 3.4 times (p = 0.0001), respectively; cholesterol — by 1.2 times (p = 0.0425) in both groups, with a tendency to increase; VLDL — by 1.8 times (p = 0.0010) and 2.5 times (p = 0.0022), respectively; and a decrease in the median HDL content by 1.7 times (p = 0.0016) and 1.9 times (p = 0.0008), respectively. The detected changes, in turn, led to a statistically significant increase in the median AC in groups I and II by 2.2 (p = 0.0036) and 2.5 times (p = 0.0007), respectively (Table 1).

It was found that patients of group I had a statistically significant increase in the TG/Gly ratio by 1.7 times. The median value was 0.21 (0.16; 0.30) compared to the control group:

<table>
<thead>
<tr>
<th>Biochemical indicator</th>
<th>Controls (n = 20)</th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/l</td>
<td>4.28 (4.01; 4.55)</td>
<td>4.98 (4.36; 5.62)*</td>
<td>4.49 (4.28; 5.29)</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>0.54 (0.50; 0.62)</td>
<td>1.36 (0.90; 1.80)***</td>
<td>1.86 (1.15; 2.26)***</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.64 (1.42; 1.68)</td>
<td>0.96 (0.80; 1.12)***</td>
<td>0.83 (0.72; 0.97)***</td>
</tr>
<tr>
<td>LDL-C, mmol/l</td>
<td>2.63 (2.56; 2.97)</td>
<td>3.33 (2.83; 4.20)**</td>
<td>2.88 (2.55; 3.63)</td>
</tr>
<tr>
<td>VLDL-C, mmol/l</td>
<td>0.34 (0.17; 0.36)</td>
<td>0.62 (0.41; 0.82)**</td>
<td>0.85 (0.53; 1.03)**</td>
</tr>
<tr>
<td>AC</td>
<td>1.89 (1.74; 2.61)</td>
<td>4.13 (3.16; 5.44)***</td>
<td>4.79 (3.7; 5.58)**</td>
</tr>
</tbody>
</table>

Notes: the probability of changes between the indicators in groups I and II compared to the control group: * — p < 0.05; ** — p < 0.01; *** — p < 0.001.
0.11 (0.10; 0.13) (p < 0.05), while in patients of group II, an even greater increase in this index by 2.4 times was found, with a median of 0.35 (0.21; 0.45) (p < 0.05) (Fig. 2).

There is an independent relationship between TG/HDL-C ratio and NAFLD, which is not associated with obesity and dyslipidemia, so TG/HDL-C level can be used as the best predictor of NAFLD. Our results showed an increase in the median of TG/HDL-C ratio in patients of group I by 3.6 times (p < 0.05), which was 1.14 (0.76; 2.06), compared to the healthy controls: 0.32 (0.28; 0.34), and a statistically significant increase in group II by 6.8 times (p < 0.05), where the median value was 2.20 (1.12; 3.19) (Fig. 3).

The difference in the levels of TG/Gly and TG/HDL-C between groups I and II was a statistically significant increase by 1.5 (p = 0.038) and 1.9 times (p = 0.035), respectively.

In patients with NAFLD, statistically significant differences in carbohydrate metabolism in terms of insulin and HOMA-IR were found: in group I, an increase by 2.5 times (p < 0.05), in group II — by 2.4 times (p < 0.05), and there was a significant increase in HOMA-IR compared to the control group (Table 2).

The conducted studies showed that HOMA-IR in 84.2% of patients in group I and 94.4% in group II was higher than 3.0. The medians were significantly different from the data in the control group, namely, there was an increase by 3.1 times (p < 0.05) in group I and by 3.0 times (p < 0.05) in group II.

**Discussion**

One of the most well-known mechanisms of disease progression in NAFLD is steatosis. When the ability of hepatocytes to synthesize TG exceeds their ability to get rid of them, they accumulate inside them as fat. Although TG are not toxic themselves, their precursors such as VLC and other metabolic byproducts like reactive oxygen species are toxic to hepatocytes. The accumulation of these byproducts is known as lipotoxicity [17]. Due to impaired lipid metabolism, patients with NAFLD experience intra- and intrahepatic lipid accumulation such as increased hepatic consumption of VLDL and VLDL synthesis, dysregulation of TG export, and decreased HDL and cholesterol levels. Inflammation also contributes to the production of cytokines, intestinal products (e.g., lipopolysaccharide), and hepatotoxic mediators that can worsen NAFLD if they affect hepatocytes [7, 10].

In patients with NAFLD who had SARS-CoV-2, lipid metabolism disorders worsen, namely, the content of VFAs in the blood serum can both increase and decrease, the synthesis of fatty acids in the liver with abnormal β-oxidation increases, and a greater percentage of TG in the liver is formed from absorbed non-esterified fatty acids. The metabolism of FFAs is as important for viruses as it is for eukaryotic cells. However, since viruses do not have enzymatic pathways to synthesize FFAs, they separate the machinery from host cells and manipulate the host cell’s lipid metabolism to facilitate their assembly and replication without considering the consequences for the host. One of the virus strategies is to reprogram the host’s fatty acid metabolism to provide the necessary fatty acid molecules for the synthesis of virion replication membranes. Isomers of SREBP are involved in this metabolic reprogramming. As an example of such reprogramming, Middle East respiratory syndrome coronavirus and flavivirus can manipulate host cellular lipid metabolism and reprogram de novo the SREBP-dependent lipogenesis pathway to enable its replication. Analysis of plasma lipids from patients with Ebola virus disease has shown that lipids are important for structural components of the viral membrane, signaling molecules, and as a source of energy. This

![Figure 2 — Distribution of absolute TG/Gly values in patients with NAFLD with an immune response to SARS-CoV-2, Me (Q1; Q3)](image)

![Figure 3 — TG/HDL-C levels in patients with NAFLD with an immune response to SARS-CoV-2, Me (Q1; Q3)](image)

<table>
<thead>
<tr>
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<th>Group II (n = 18)</th>
<th>Controls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, μIU/ml</td>
<td>27.7 (19.3; 39.1)*</td>
<td>25.9 (20.0; 38.7)*</td>
<td>11 (2.3; 19.4)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.3 (4.75; 5.35)</td>
<td>5.5 (4.6; 5.6)</td>
<td>4.4 (4.1; 4.7)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>6.5 (4.15; 8.4)*</td>
<td>6.2 (4.5; 8.9)*</td>
<td>2.1 (1.4; 2.8)</td>
</tr>
</tbody>
</table>

**Table 2 — Indicators of carbohydrate metabolism in patients with NAFLD with an immune response to SARS-CoV-2, Me (Q1; Q3)**

**Note.** * — p < 0.05 compared to the control group.
suggests that altering the lipid homeostasis of host cells is a strategy of the virus to create a suitable environment for replication [9, 10].

The progression of metabolic disorders associated with obesity such as IR and dyslipidemia, inflammation contribute to the development of cardiovascular disease, type 2 diabetes mellitus, fatty liver disease, which significantly impair the quality of life, reduce life expectancy and increase mortality at a young age [7, 11, 18]. According to current concepts, the inducer of metabolic disorders is low-grade inflammation induced by adipocyte dysfunction in the context of obesity. Metabolically active cells such as adipocytes secrete numerous anti-inflammatory cytokines and chemokines, adiponectin, and leptin in obesity [19]. According to many authors, TNF-α and interleukin-6 play the most important role in the pathogenesis of NAFLD among inflammatory mediators [11, 12, 13, 20]. They are non-adipose tissue-specific cytokines but have a significant impact on the metabolism of fats and carbohydrates [21]. However, other studies have shown that severely malnourished individuals show signs of systemic inflammation with increased serum interleukin-6 concentrations compared to healthy individuals [22]. Increased production of TNF-α and interleukin-6 is believed to play a special role in the pathogenesis of IR. Many researchers consider TNF-α as a mediator of IR in obesity. There is evidence of the ability of TNF-α to enhance cell necrosis and apoptosis [21, 23].

In our study, it was determined that carbohydrate metabolism disorders in the form of IR are typical for patients with NAFLD. It is known that IR triggers visceral fat lipolysis and oxidation of free fatty acids by the liver, causing gluconeogenesis and fatty infiltration of hepatocytes [10, 19]. IR also leads to atherosclerotic changes in arterial vessels, resulting in changes in their elastic and elastic properties [21, 24].

Conclusions

1. Patients with NAFLD with an immune response to SARS-CoV-2 have lipid metabolism disorders. Thus, dyslipidemia manifested by a significant increase in the median TG content in groups I and II by 2.5 and 3.4 times (p = 0.0001), respectively; cholesterol — by 1.2 times (p = 0.0425) in both groups, with a tendency to increase; VLDL — by 1.8 (p = 0.0110) and 2.5 times (p = 0.0022), respectively, and a decrease in the median HDL content by 1.7 (p = 0.0016) and 1.9 times (p = 0.0008), respectively. The detected changes led to a statistically significant increase in the median atherogenicity coefficient in groups I and II, by 2.2 (p = 0.0036) and 2.5 times (p = 0.007).

2. It has been found that patients with NAFLD with an immune response to SARS-CoV-2 have carbohydrate metabolism disorders. An increase in the HOMA-IR was found, by 3.1 times (p < 0.05) in group I and by 3.0 times (p < 0.05) in group II compared with controls, indicating the development of insulin resistance in patients with NAFLD.

3. Patients in groups I and II had a statistically significant increase in the following ratio: TG/Gly by 1.7 and 2.4 times, TG/HDL-C in by 3.6 and 6.8 times (p < 0.05). The detected changes were more pronounced in patients of group II.

4. TG/Gly and TG/HDL-C ratio should be included in the diagnostic algorithm for screening metabolic disorders in patients with COVID-19 who are overweight or obese.

References


Метаболічні порушення в пацієнтів із неалкогольною жировою хворобою печінки з імунною відповіддю на SARS-CoV-2