Study the Metabolism of Fatty Acids in Sera of COVID-19 Patients in Duhok City/Iraq

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Abstract
Background and objectives: *COVID-19 has been increasingly reported with derangement of biological pathways which might ultimately affect the metabolism. We sought to identify the impact of COVID-19 on metabolic pathways.*
Methods: *The study dealt with two groups of people, the first group was (25) men with COVID-19, their ages ranged between (40-60) years, and were undergoing chemotherapy. The second group included (25) healthy men who did not have any apparent disease as a control group, where blood was drawn from the vein area, then the blood serum was separated by a centrifuge, and then the serum was divided into two parts. The first part was used to measure the different biochemical variables that have a Relationship to lipid metabolism of different types in blood serum, while the second part was preserved until analysis of fatty acids. The serum lipids were extracted using organic solvents, and they were separated from each other using thin-layer chromatography (TLC). Then the percentage of saturated and polyunsaturated fatty acids (mono and poly) was measured using gas chromatography (CGC) for the three fractions of blood serum (CE), (TG), (PL) after the fatty acid re-esterification process. Results: It was observed that there are differences between the percentage of fatty acids of various types between the group of patients and the control group and in the different parts of the blood serum lipids, and this indicates the effect of fatty acids in the development of COVID-19 diseases, including risk factors for cardiovascular disease through the difference and imbalance that occurs in the percentage, especially of poly-saturated fatty acids (PUFA). Conclusion: The derangement in metabolic parameters have been confirmed and taken to consideration for future direction of treatment and challenges of viral infections.*
Keywords: *COVID-19, Fatty acids, Cholestrolester, Phospholipid.*

INTRODUCTION

In recent years, the world has seen a significant increase in cases of morbidity and mortality due to coronavirus infections (Darweesh et al., 2021; Haider et al., 2022). The specific virus responsible for the current pandemic of Coronavirus disease (COVID-19), was initially known as novel coronavirus 2019 (nCoV-2019) but is now referred to as acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Ejaz H et al., 2020). This highly perplexing infection has been found to result in a distinctive set of metabolic and clinical changes in those it affects (Petrakis et al., 2020). The primary form of COVID-19 can cause a decrease in white blood cells, a decrease in lymphocytes, and an increase in levels of the protein C-reactive protein (CRP). As the disease progresses, the levels of white blood cells, creatine kinase, and creatinine may also increase (Rando et al., 2021).
The nutritional status of the individual (McFann et al., 2021) and drug profile (Zainal & Merkhan, 2022) plays a crucial role in their ability to fight off viral infections. Multiple studies have revealed that those who are malnourished are more prone to a variety of infections (Soriano et al., 2022). Maintaining a proper and well-rounded diet is essential in protecting the body's defence system and boosting its performance (H. G. Zhang et al., 2023). A lack of essential

nutrients can significantly impact both the body's immune response and the harmful abilities of viruses (Mandal et al., 2021; McFann et al., 2021). When these key nutrients are lacking, the body experiences heightened levels of oxidative stress which can alter the genetic composition of viruses, potentially transforming them from harmless to highly virulent pathogens (Soriano et al., 2022). Omega-3 polyunsaturated fatty acids (n3-PUFAs) play a crucial role in controlling inflammation and strengthening acquired immune responses, effectively bolstering antiinflammatory reactions (Boroughs & DeBerardinis, 2015). Recent research has demonstrated that n3-PUFAs, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and αlinoleic acid (ALA), can increase the stability of cell membranes, regulate immune function, suppress excessive inflammatory reactions, and decrease the likelihood of systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), and infection-related complications (B. Wang & Tontonoz, 2019).

The SARS-CoV virus attaches lipid chains to specific cysteine spots near its transmembrane parts (Beilstein et al., 2017). This process, known as cysteine palmitoylation, adds palmitate (C16:0), stearate (C18:0), or arachidonate (C20:0) to cysteine residues, making the protein more hydrophobic and increasing its affinity for cellular membranes and adjacent proteins or domains (J.-Z. Zhang, 2003). Apart from tethering to the membrane, this modification also facilitates protein movement between different membrane compartments (Booth, 2003). Proteins that undergo palmitoylation can be divided based on the location of the lipid addition, including those with palmitoylated cysteines near or within the transmembrane sequence (within 20 amino acids), at the carboxyl-terminus or near the amino-terminus, and possessing the MGC motif (Louca et al., 2021; Mandal et al., 2021). Although there are many mechanisms and methods that cause the transmission of COVID-19 in the severe stages, which are not yet precisely known, many studies and evidence indicate that the high level of inflammation contributes negatively to the increase in infection and in a severe manner and causes acute infection, as a number of studies have proven the existence of a link between obesity and a higher level of pro-inflammatory factors. Fatty acids are among the main regulatory factors for many inflammatory pathways in the human body, as saturated fatty acids are considered the worst in this aspect and participate more in systemic inflammation. Several studies have found that abnormalities in lipid metabolism, fatty acid synthesis, and metabolism lead to increased susceptibility to COVID-19 infection. Other studies have shown that the number of inflammatory receptors for a specific number of fatty acids contributes significantly to increased vascular permeability and increased pulmonary inflammation, leading to an increased likelihood of developing acute respiratory distress syndrome. The level of fatty acids in the blood serum determines the severity of COVID-19 infection. (Stromberg et al., 2022),(Sun et al .,2022), (Lampova et al., 2022).

MATERIALS AND METHODS

Subjects and blood sampling: The study enrolled two groups of people in Dohuk city, the first group (25) was men with COVID-19, their ages ranged between (40 and 60) years, the patients' samples were from people who were recently infected with the COVID-19 virus and were diagnosed in the hospital and did not receive any chemotherapy when the blood serum was drawn, where samples were taken after the approval of the patients and the hospital administration. The second group included (25) healthy men as a control group. Blood was withdrawn, the serum separated, and then the serum was divided into two parts. The first part was used to measure the different biochemical variables that have a Relationship of lipid metabolism of different types in blood serum, while the second part was preserved until the analysis of fatty acids.

Lipid analysis: The blood serum parts were separated using the thin layer chromatography technique, through the use of the special vessel for the technique, in addition to a silica gel plate with dimensions of (20 * 20) cm, through the use of a mobile phase consisting of (hexane: ether: formic acid) (80:20:2), where the blood serum spot is placed After separating the proteins using (ethanol: methanol) on the starting line of the plate, and the process of passing the mobile phase inside the container for (45) minutes to the finish line, then the fat spots are endorsed using a dye (2',7'-dichlorofluorescein) (B. Wang & Tontonoz, 2019). After that, the process of scraping the spots is done, and then the process of re-esterification of the fatty acids in the blood serum parts is performed using the method (BF3/methanol) and then the percentage of fatty acids in the separated parts is measured using a capillary gas chromatography device of the Shimadzu type 2014 (Beilstein et al., 2017; J.-Z. Zhang, 2003). The gas chromatogram column is made of metal (copper) or glass coil, and its length ranges between (10-100) m. Device type: SHIMADZU CORPORATION _2010_JA PAN.,Colum name:SP2480, Colum length: 50 m, Colum diameter: 0.25mm, Temperature: (70-250) ^{0} C,Percentage gas: nitrogen gas N_2, Measurment time: 25 min.Propion of fatty acid in spermatozoa was estimated through the injection of (1) µl of the sample to the (CGC).

RESULTS AND DISCUSSION

The percentage of fatty acids in phospholipids: The percentage of fatty acids was measured using capillary gas chromatography (CGC) by comparing the results with a sample consisting of (18) standard fatty acids.

The percentage of SFA in the phospholipids section of patients' blood was significantly higher than that of the control group because SFA negatively affects patients by making it easier for the virus to enter host cells (Table 1). The reason for this may be that (SARSCov-2) enhances the activity of the synthesis of saturated fatty acids, particularly palmitic acid, by organizing and arranging the genes responsible for the formation By greasing up cell walls through restricting to cysteines in the spike and shut proteins SFA. The outcomes in this study demonstrated a huge expansion in the level of unsaturated fats (MUFA) in the phospholipids part of serum in patients contrasted with the benchmark group (Table 1). The justification for this might be because of the way that monounsaturated unsaturated fats intervene hostile to viral movement by attempting to break and obliterate the external shell of the infection, including the Coronavirus and influenza, as oleic fatty acid has a strong correlation with the severity and development of the disease and plays a major role in the process of the development of the Coronavirus SFA. On the other hand, the study's findings showed that patients' blood levels of PUFA in the phospholipids section were significantly higher than those of the control group (Table 1). This could be because polyunsaturated fatty acids have a structural function that keeps the virus from infecting the host by binding the spike protein to the virus's envelope and locking it in a position that inhibits interaction with ACE2. Studies indicate that PUFA interferes with the receptor in the field of Binding the virus to the protein (SARS-CoV2) and preventing interaction with the receptors. In advanced cases, the consumption of PUFA is very high due to its role as an anti-inflammatory SFA.

Table 1. Fatty acids in PL part

Fatty acid	Control (n=20)	Case (n=20)	P value
C16:0	4.66±0.213	9.40±0.140	0.051
C18:0	5.31±0.573	14.51±0.065	0.001
C23:0	1.32±0.062	2.44±0.080	0.030
Total	11.29±0.848	26.35±0.285	0.001
C18:1 Trans	0.177±0.025	1.23±0.021	0.71
C18:1 cis	0.92±0.013	2.67±0.120	0.004

C20:1	0.265±0.062	0.525±0.012	0.023
Total	1.362±0.100	4.425±0.153	0.001
C18:3	2.134±0.921	9.65±0.261	0.001
C18:2	1.210±0.024	1.13±0.017	0.023
Total	3.344±0.945	10.78±0.278	0.001

The percentage of fatty acids in triglyceride: The study's findings also showed that, in comparison to the control group, patients' blood serum triglyceride fraction had significantly more SFA (Table 2). The reason for this may be due to the metabolism of triglycerides has a very large defect in COVID-19 patients, which leads to a significant increase in patients, and thus the percentage of saturated fatty acids increases in this part of the blood serum, as high triglycerides are one of the components of the formation and development of inflammation because the particles rich in fats The triglyceride in serum increases local inflammation.

When comparing the patients' group to the control group, the study's findings revealed a noteworthy rise in the concentration of monounsaturated fatty acids (MUFA) in this section of the blood serum (Table 2). The justification for this might be because of the job of food, whose impact is obvious in this piece of the blood serum, as the unevenness that happens in digestion enormously influences the digestion of fats and carbs as far as their job in the vital energy creation process in numerous metabolic pathways, including Fat digestion (Yang et al., 2012). The results of this study also indicated a high increase in the level of polyunsaturated fatty acids (PUFA) in this part of the blood serum when comparing the group of patients with the control group (Table 2). The reason for this may be due to a significant defect in the genes in the liver related to the process of triglyceride metabolism and this gene (PPARa), which controls the process of lipid metabolism, especially unsaturated fatty acids. Where a significant increase in the level of the gene (PPARa) was observed in patients in previous studies, and it is inversely proportional to the level of polyunsaturated fatty acids, which in turn is considered a risk factor in the development of symptoms of hypothyroidism in terms of cardiovascular disease. Hematologic) Souza *et al.*, 2011). The reason for the low level of polyunsaturated fatty acids may also be due to the role of hepatic lipase, whose activity increases as beta-oxidation processes increase, This causes the amount of polyunsaturated fatty acids, particularly EPA and DHA, to rise. As a result, the amount of polyunsaturated fatty acids in this section of the serum blood drops (Soukup, 2014).

Table 2. Fatty acids in TG part

Fatty acid	Control (n=20)	Case (n=20)	P value
C16:0	5.33±0.821	10.30±0.092	0.001
C18:0	3.84±0.140	16.39±0.104	0.001
C23:0	1.21±0.052	2.89±0.064	0.030
Total	10.38±1.01	29.58±0.26	0.001
C18:1 Trans	0.72±0.33	2.56±0.211	0.005
C18:1 cis	0.93±0.05	4.21±0.012	0.004
C20:1	2.33±0.10	0.86±0.014	0.028
Total	3.98±0.48	7.63±0.237	0.050
C18:3	3.76±0.821	10.56±0.182	0.001
C18:2	1.85±0.012	1.98±0.039	0.226
Total	5.61±0.833	12.54±0.221	0.001

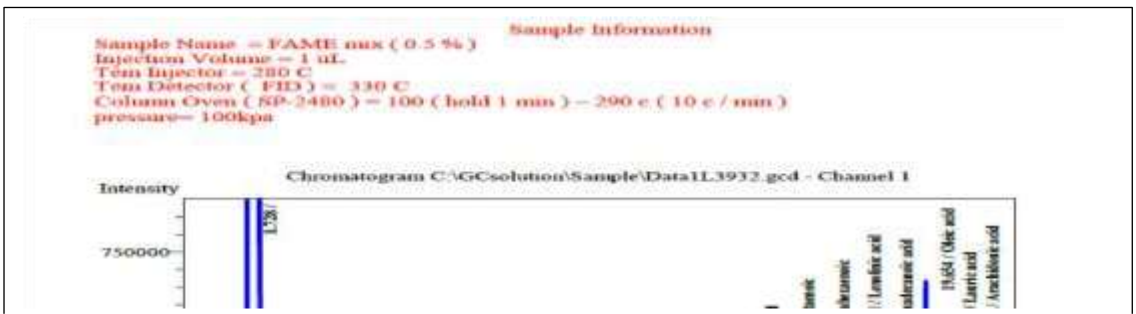
The percentage of fatty acids in Cholesterolester: The a significant increase in polyunsaturated fatty acids (PUFA) and a decrease in monounsaturated fatty acids (MUFA) in this portion of the blood serum when comparing the patient group to the control group (Table 3). These findings also showed a significant increase in the percentage of SFA in the cholesterol ester fraction. The reason for this may be COVID-19 patients have a major defect in various metabolic processes, especially fats and their metabolism. Therefore, it is observed that there is an increase in the level of blood serum cholesterol, as well as an increase in the percentage of long-chain saturated and unsaturated fatty acids (T. Wang et al., 2022), as the metabolic defect greatly affects the transmission of infection and spread of the disease through the host cell feeding the virus (Miller et al., 2022). Polyunsaturated fatty acids produce large amounts of oxylipins (prostaglandins and leukotrienes), which help fight inflammation. The reason may also be due to the role of the imbalance that occurs in fatty acid metabolism in Corona patients and the association with the platelet-activating factor, which can contribute to an increase in the percentage of PUFA and therefore, depending on that, the immune response is weak and irregular, and clotting processes occur as a result. Blood in patients, as well as the cytosolic (PLA2) pathway, is activated and then affects thromboxane and platelets, as recent studies have indicated an effect of PUFA and (PLA2) as the two main elements in the occurrence and development of Corona disease.

Table 3. Fatty acids in CE part

Fatty acid	Control (n=20)	Case (n=20)	P value
C16:0	4.41±0.121	8.42±0.138	0.001
C18:0	6.32±0.324	13.18±0.104	0.001
C23:0	1.1±0.054	2.05±0.077	0.013
Total	11.83±0.499	23.65±0.269	0.001
C18:1 Trans	0.76±0.621	1.67±0.021	0.143
C18:1 cis	1.20±0.367	2.11±0.011	0.640
C20:1	2.73±0.631	0.29±0.026	0.058
Total	4.69±1.619	4.07±0.058	0.900
C18:2	5.54±0.976	9.13±0.026	0.006
C18:3	2.34±0.988	1.11±0.029	0.004
Total	7.88±1.955	10.24±0.055	0.001

The high percentage of PUFA in the three parts of blood serum in Corona patients is considered a negative indicator of the severity of the disease, as it can be used in diagnosing cases of infection with the disease, as many recent studies have found a correlation between the percentage of PUFA. With the severity of infection with the virus, knowing that some PUFA, especially DHA work to reduce the severity of infection and symptoms of the disease through its role in causing immune changes by increasing the immune response against the virus and avoiding tissue damage.

Other studies indicated that there is an interaction between the body mass index, white blood cells, some types of circulating proteins, smoking, and the percentage of PUFA. This interaction was large in obese people (high fat), and they were more susceptible to infection and its severity



than people of normal weight, and to the development of diseases. Including heart disease, hypertension, diabetes, and cancer, as fatty acids, especially (n-3) play an essential role in solving the problem of inflammation and tissue repair through derived prostaglandins. Some studies have noted that fatty acids (n-3) reduce the process of entry of the virus into host cells by reducing the process of binding to the glycoprotein (SARS-CoV-2) and reducing its interaction with the receptor (ACE2) as this interaction and binding leads to the emergence of cells (Huh-7) derived from the liver and is considered one of the lines. Cancer during the development of Corona disease. It has been observed that taking nutritional supplements that contain fatty acids (LA)(AA) reduces the multiplication and spread of the virus, especially (HCOV-229E) and also prevents and reduces the occurrence of coronavirus syndrome (MERS-CoA).

Figure showing the identification of standard fatty acid using capillary gas chromatography

CONCLUSION

The study results indicate a significant impact of COVID-19 on various biochemical parameters in patients. These changes include a significant increase in the levels of saturated fatty acids in the three parts of the serum compared to healthy individuals, which constitutes a high risk factor for increased inflammatory receptors. The results also indicate the presence of abnormalities in terms of significant increases or decreases in the levels of monounsaturated and polyunsaturated fatty acids when comparing the patient group with the control group. These findings enhance our understanding of the physiological changes associated with COVID-19 and may impact patient care and treatment strategies. Further research is needed to explore the mechanisms underlying these biochemical changes, their clinical significance, and their relationship with the levels of various fatty acids in the management of COVID-19.

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