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Investigation of organic acid levels as a prognostic indicator in Covid-19 positive patients

Covid-19 pozitif hastalarda prognostik bir göstergesi olarak organik asit düzeylerinin araştırılması

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ABSTRACT

Objective: Ever since Covid-19 outbreak in Wuhan, China, medical scientists actively strive to contribute in the diagnosis and management of the disease. For this purpose, many markers have been studied so far for the follow-up and prognosis of the disease, apart from these routine tests, the number of targeted and untargeted metabolomic studies have been growing gradually. Organic acids are the intermediate products in various metabolic pathways. Abnormal urinary organic acids can be present in amino acid and carbohydrate metabolism disorders, mitochondrial fatty acid beta oxidation disorders, and some mitochondrial oxidative phosphorylation defects. The aim of this study is to evaluate the organic acids detected in the urine due to the metabolic processes that may be affected by hypoxia in Covid-19 patients who develop clinically mild, moderate and severe pneumonia, to determine their relationship with the disease severity and to compare the results with the control group.

Methods: A total of 120 patients with the clinical spectrum of SARS-CoV-2 infection as mild, moderate and

ÖZET

Amaç: Covid-19 salgınından bu yana, tıp bilim adamları aktif olarak hastalığın teşhisine ve tedavisine katkıda bulunmaya çalışmaktadırlar. Bu amaçla bugüne kadar hastalığın takibi ve prognozu için birçok belirteç çalışılmıştır. Rutin testlerin dışında hedefli ve hedefsiz metabolomik çalışmaların sayısı giderek artmaktadır. Organik asitler, çeşitli metabolik yollardaki ara ürünlerdir. Anormal üriner organik asit düzeylerine, amino asit ve karbonhidrat metabolizması bozukluklarında, mitokondriyal yağ asidi beta oksidasyon bozukluklarında ve bazı mitokondriyal oksidatif fosforilasyon kusurlarında rastlanabilir. Bu çalışmanın amacı, klinik olarak hafif, orta ve şiddetli pnömoni gelişen Covid-19 hastalarında hipoksiden etkilenebilecek metabolik süreçler nedeniyle idrarda saptanan organik asit düzeylerini değerlendirmek, hastalık şiddeti ile ilişkisini belirlemek ve sonuçları kontrol grubu ile karşılaştırmaktır.

Yöntem: Bu çalışmaya SARS-CoV-2 enfeksiyonunun klinik spektrumu Dünya Sağlık Örgütü Covid-19 hastalık

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severe according to the WHO Covid-19 disease severity classification were included in the present study. First morning urine samples taken inpatient setting were aliquoted and frozen immediately until the analysis day. Urine organic acid levels were measured using Gas chromatography-mass spectrometry.

Results: Among 104 organic acids, pyruvic acid, lactic acid, 2-hydroxybutyric acid levels were significantly higher in the severe disease group (p=0.006, p<0.001, p<0.001, respectively), citric acid and homovanilic acid (HVA) levels were significantly lower in all disease groups (p<0.001), α-ketoglutaric acid, vanillylmandelic acid and succinic acid levels were significantly lower in the severe and moderate disease group (p<0.001), ß-hydroxybutyric acid was significantly higher in the severe and moderate disease group p(<0.001), oxalic acid was significantly higher in the moderate disease group compared to the control group (p=0.041). The binary logistic regression model included, α-ketoglutaric acid, succinic acid, and HVA. It was statistically significant with x^2 = 98.680; p < 0.001.

Conclusion: α-ketoglutaric acid, succinic acid and HVA were independent predictors of the disease severity, measurement of these spesific metabolites might facilitate the developments of novel therapies and may improve responses to currently available therapies with the ease of prognosis prediction of Covid-19.

Key Words: Covid-19, severity, organic acids, urine, GC-MS

şiddeti sınıflandırmasına göre hafif, orta ve şiddetli olarak kabul edilen toplam 120 hasta dahil edildi. Yatan hastalardan alınan sabah ilk idrar örnekleri alikotlara ayrıldı ve analiz gününe kadar dondurulduru. İdrar organik asit seviyeleri, Gaz kromatografisi-kütle spektrometresi kullanılarak ölçüldü.

Bulgular: 104 organik asit arasında piruvik asit, laktik asit, 2-hidroksibütirik asit düzeyleri ağır hastalık grubunda anlamlı olarak yüksekti, (sırasıyla p=0,006, p<0,001, p<0,001), sitrik asit ve homovanilik asit (HVA) düzeyleri ise tüm hastalık gruplarında anlamlı olarak daha düşüktü (p<0.001), α-ketoglutarik asit, vanililmandelik asit ve süksinik asit seviyeleri, şiddetli ve orta hastalık grubunda anlamlı olarak düşüktü(p<0.001), ß-hidroksibutirik asit, şiddetli ve orta hastalık grubunda anlamlı olarak daha yüksekti p(<0,001), oksalik asit orta hastalık grubunda kontrol grubuna göre anlamlı olarak yüksekti (p=0,041). İkili lojistik regresyon modeli, a-ketoglutarik asit, süksinik asit ve HVA'yı içeriyordu. x^2 = 98.680 ile istatistiksel olarak anlamlıydı; p < 0.001.

Sonuç: α-ketoglutarik asit, süksinik asit ve HVA, hastalık şiddetinin bağımsız belirteçleri olarak bulundu. Bu spesifik metabolitlerin ölçümü, yeni tedavilerin geliştirilmesini kolaylaştırabilir ve Covid-19'un prognoz tahminini kolaylaştırarak mevcut tedavilere verilen yanıtları iyileştirebilir.

Anahtar Kelimeler: Covid-19, şiddet, organik asitler, idrar, GC-MS

INTRODUCTION

Coronavirus disease 2019 (Covid-19) is an ongoing worldwide pandemic, affecting 767 million people and causing death of more than 6.9 million people to date (1). The major clinical signs are fever, cough and fatigue, and also lymphopenia, pneumonia, multi-organ failure and death can be seen in severe and uncontrolled cases. Although most of the cases are mild, hypoxia plays an important role in the formation of undesirable clinical results particularly in severe cases.During hypoxia, along with the physiological changes for returningoxygen level to normal, there are also changes at the molecular level such as the induction of Hypoxia-inducible factor 1-α (HIF 1-α). HIF 1-α is the main regulatory molecule of oxygen homeostasis in mammals and has crucial roles on metabolism during hypoxic conditions. HIF 1-α acts as

a factor to increase the transcription and translation of most glycolytic enzymes and Glucose transporter 1(GLUT1) and Glucose transporter 3(GLUT3) to overcome the energy shortagedue to disruption of the oxidative phosphorylation as the main source of ATP production. Asanaerobic glycolysis increases, pyruvate is converted to lactate in order to maintain NAD+/NADH equilibrium. Other α-keto-acid oxidases, such as malate dehydrogenase, also help to replace the cytosolic NAD+pool in a hypoxic environment. HIF 1-α also affects metabolic processes such as fatty acid synthesis, sterol synthesis, triglyceride synthesis and ß-oxidationof fatty acids in lipid metabolism (2, 3)

Organic acids are water-soluble compounds that do not contain amino groups and contain one or more carboxyl groups as well as functional groups. In our body, they appear as intermediate products in various metabolic pathways. More than 500 organic acids have been identified in the urine, and although these can be seen in the urine of clinically normal individuals; a single organic acid or combinations of organic acids might increase when several metabolic pathways are blocked. Abnormal urinary organic acids can be present in amino acid and carbohydrate metabolism disorders, mitochondrial fatty acid beta oxidation disorders, and some mitochondrial oxidative phosphorylation defects. Besides, urinary organic acid levels may increase in sterol, neurotransmitter, vitamin, purine and purimidine metabolism disorders (4). Along with genetic and metabolic diseases, it has been reported that organic acid levels in the urine are increased in certain pathological conditions such as ketosis associated with renal dysfunction types (5).

Ever since Covid-19 outbreak in Wuhan, China, medical scientists actively strive to contribute in the diagnosis and management of the disease. For this purpose, many markers have been studied so far for the follow-up and prognosis of the disease, including coagulation factors, hematological markers, inflammation markers, cardiac markers, hepatic markers, muscle markers, renal markers and electrolytes. Among these markers, white blood cell, neutrophil, lymphocyte, platelet count, C-reactive protein (CRP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), D-dimer, fibrinogen and procalcitonin levels have been associated with the prognosis of the disease (6, 7). Apart from these routine tests, the number of targeted and untargeted metabolomic studies have been growing gradually. Amino acid metabolism, tricarboxylic acid cycle (TCA) and energy metabolism, NAD+ metabolism, primary bile acids, purine and purimidine metabolism were evaluated and different metabolites were found to be associated with the severity of the disease. Among these studies, kynurenine pathway metabolites were the most studied (8). The aim of this study is to evaluate the organic acids detected in the urine due to the metabolic processes that may be affected by hypoxia in Covid-19 patients who develop clinically mild, moderate and severe pneumonia, to determine their relationship with the disease severity and to compare the results with the control group.

MATERIAL and METHOD

Patient selection

Adult patients admitted to the hospital with clinical signs and symptoms associated with Covid-19 and hospitalized at Infectious Diseases Clinic with confirmed diagnosis by detecting SARS-CoV-2 RNA in oro-nasopharyngeal swab samples were participated in the present study. A total of 120 patients with the clinical spectrum of SARS-CoV-2 infection as mild, moderate and severe according to the WHO Covid-19 disease severity classification were included in the present study. Based on this classification, mild patient group was composed of patients without evidence of pneumonia or hypoxia (n=40) whereas moderate patient group was composed of patients with signs and symptoms of pneumonia without signs of severe pneumonia (n=40). The patients with pneumonia and any of the following; > 30 breaths/min; severe respiratory distress; or SpO2 < 90%at room air were classified in severe patient group (n=40). Additionally,

the control group consisted of volunteers without any clinical signs and symptoms associated with Covid-19(n=30) were included in the study. In the patient group, 15 patients had hypertension, 10 patients had diabetes mellitus (DM), 4 patients had coronary artery disease, 2 patients had lung disease, 2 patients had hypertension and chronic kidney disease, 1 patient had lung and kidney disease, 7 patients had hypertension and DM, 1 patient had hypertension and DM. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Hospital and written informed consent was obtained from the patients.

Sample Collection and Analyses

First morning urine samples taken inpatient setting were aliquoted and frozen immediately until the analysis day. All samples were thawed and vortexed before analysis. All analyses were performedin Medical Biochemistry Laboratory. The determination of creatinine content of the urine samplewas performed in Atellica autoanalyser (Siemens Healthineers, Erlangen, Germany) according to manufacturer's instructions using original kits based on Jaffe method. The analysis of organic acids (OA) by gas chromatography-mass spectrometer (GC-MS) after ethyl acetate extraction/oxime-trimethylsilyl derivatization was carried out for profiling urinary organic acids as described in the literature (9). The derivatized extract was injected in the GCMS-QP2010 SE (Shimadzu Co., Kyoto, Japan)for analysis. Electron impact mass spectrometry was performed in the scan mode with a mass range between m/z 40 and 600. The individual peaks of the organic acids were compared with the library for peak identification and the amount was obtained by ratio to the 4-phenylbutyric acid which was used as pure standard.All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Samples were introduced into the column via a splitless injector at 280°C. The initial oven temperature was kept at 100°C for 4 minutes and then programmed to rise 4°C/min to a final temperature of 320°C. This temperature was maintained for 10 minutes. The column was TRB 5MS 30mx0.25mmx1ul with a constant flow of helium at 1ml/minute. It was inserted directly into the ion source with an interface temperature of 280°C. The mass spectra of all GC peaks were generated by a mass spectrometer operated in the electron impact (EI) mode.

The level of 104 organic acids, whose total ion chromatogram is shown in figure 1, was measured.These organic acids were lactic acid, 2-hydroxyisobutyric acid, glycolic acid, oxalic acid, 2-hydroxybutyric acid, 3-hydroxypropionic acid, levulinic acid, pyruvic acid, 3-hydroxybutyric acid, 3-hydroxyisobutyric acid, 2-hydroxyisovaleric acid, 2-methyl-3-hydroxybutyric acid, malonic acid, 3-hydroxyisovaleric acid, 2-keto-isovaleric acid, methylmalonic acid, 4-hydroxybutyric acid, 2-hydroxyisocaproic acid, 3-hydroxyvaleric acid, acetoacetic acid, 2-hydroxy-3-methylvaleric acid , benzoic acid, acetoacetate, 2-keto-3 metil valerik asit, octanoic acid, 2-methyl-3-hydroxyvaleric acid , glycerol , acetylglycine , ethylmalonic acid, 2-ketoisocaproic acid,phenylacetic acid, succinic acid, erythro 4,5 dihydroxy hexanoate lactone, methylsuccinic acid, glyceric acid, fumaric acid, uracil, 4,5-dihydroxyhexanoic acid lactone, 2-hydroxy glutarat lakton, propionylglycine mevalonic lactone isobutyrylglycine glutaric acid, thymine, 3-methylglutaconic acid, glutaconic acid, succinylacetone-oxime, isovalerylglycine, malic acid, adipic acid, phenyllactic acid, 5-oxoproline, 3-methyladipic acid, tiglylglycine, 3-methylcrotonoylglycine, 2-hydroxyphenylacetic acid, 2-hydroxyglutaric acid, 3-hydroxy glutaric acid, 3-phenyllactic acid, 3-hydroxy-3-methylglutaric acid, 3-hydroxyphenylacetic acid, 2-keto glutaric acid, hexanoylglycine, 4-hydroxy phenyl acetic acid, phenylpyruvic acid, n-acetylaspartic acid, suberic acid, aconitic acid, orotic acid, homovanillic acid (HVA), citric acid, 3,4-dihydroxy phenylacetic

acid, hippuric acid,homogentisic acid, methylcitric acid, vanillylmandelic acid (VMA), sebacic acid, 4-hydroxyphenyllactic acid, 3-indol asetik asit, 4hydroxy-fenil purivic acid, phenylpropionylglycine, palmitic acid, 3-hydroxysebacic acid, n-acetyltyrosine, 3-hydroxy-hüppirik asit, 5-hydroxy indolasetik asit, stearic acid, suberylglycine, 3-hydroxy dodecanedioic acid, 7-hydroxy octanoic acid, succinic semialdehyde, 2-methylbutrylglycine, 2-ethyl-3-hydroxy propionic acid, octenedioic acid, azelaic acid, pyrrole-2-carboxyl-glycine, mevalonic acid, 4-hydroxy cyclohexylacetate,dihydrouracil, allo-isoleucine, n-acetyl-ileucine, n-acetylglutamic acid, 3-hydroxyphenylhydracrylic acid.

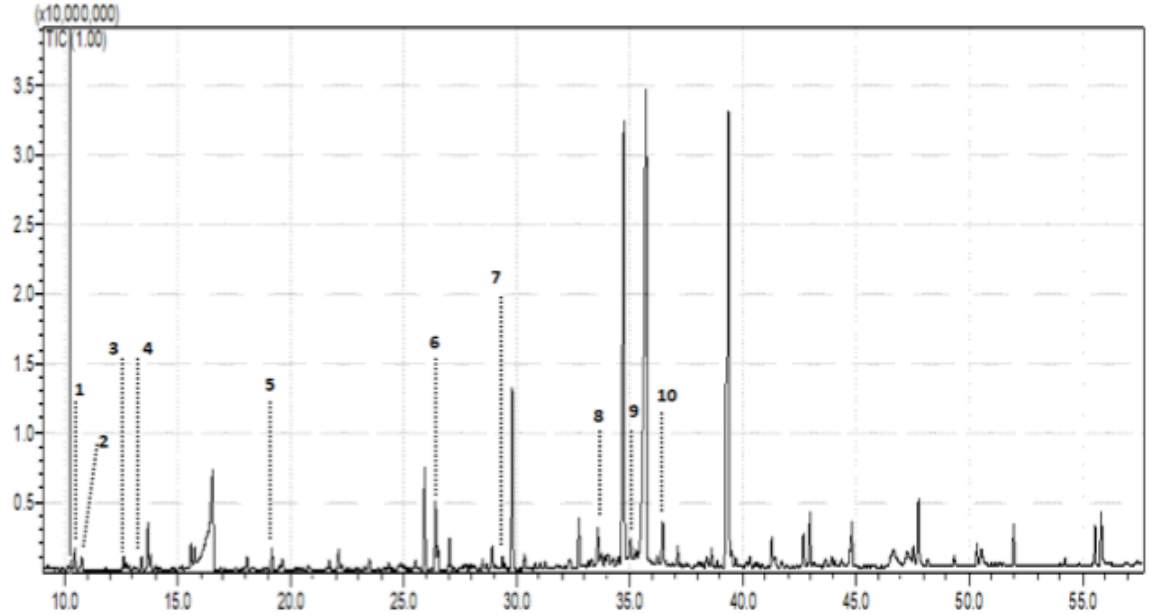

Figure 1. Typical total ion chromatogram of organic acids extracted from human urine. Numbers indicate the following metabolites.
1:Lactic acid; 2:Oxalic acid; 3:2-hydroxybutyric acid; 4: Pyruvic acid; 5:Succinic acid; 6:IS; 7: α-ketoglutaric acid; 8: HVA; 9: Citric acid; 10: VMA

Statistics

The Kolmogorov-Smirnov test was performed to check the normality of the variables. Descriptive analysis was presented using mean ± SD for normally distributed variables and median (minimum-maximum value)for non-normally distributed variables. Pairwise comparisons of groups with regard to the severity of Covid-19 using the independent samples Kruskal–Wallis test was carried out. Moreover, a binary logistic regression analysis was performed to define independent predictors of disease severity. To eliminate the effects of potential confounders, namely age, gender, and chronic disease parameters included in the model, we used the enter method, and parameters with p values greater than 0.200 were excluded for the final model with the Forward LR test. The odds ratio was calculated for significantly associated variables. Statistical significance was defined as $p < 0.05$. All statistical calculations were made using the SPSS for Windows version 26 software program (SPSS Inc., IBM Corporation, Chicago, IL).

The study was approved by the Ankara City Hospital Clinic Researches Ethics Committee (Date: 03.11.2021 and Number: E1-21-2110).

RESULTS

In our study, we evaluated 104 urine organic acids levels in severe, moderate and mild Covid-19 disease groups and compared them with the results of the control group (Table 1).We did not present unexcreted and statistically non-significant organic acid resultsamong 104 parameters. Mean±SD or median (min-max) age values of severe disease, moderate disease, mild disease and control groups were 58.8±13.5, 47.5(22-71), 32(18-71) and 40.9±20.7,respectively. Severe and moderate disease groups are different from mild disease and control groups (p<0.001).To eliminate the effects of age gap along with the other potential confounders, namely gender, and chronic disease parameters were included in the modelto define independent predictors of disease severity.

Of the patients included the study, 69 were male and 51 were female. There were 17 males and 13 females in the control group. There was no significant difference between the patient groups in terms of gender between the patient groups and the control group (>0.096).

In our study, pyruvic acid and 2-hydroxybutyric acid levels were significantly higher in the severe disease group than in all other groups (p=0.006 and p=0.001, respectively). Lactic acid levels were significantly higher in the severe disease group compared to the mild disease group and the control group (p<0.001). Citric acid and homovanilic acid levels were significantly lower in all disease groups compared to the control group (p<0.001). α-ketoglutaric acid levels were significantly lower in the severe and moderate disease group compared to the control group, in the severe disease group compared to the mild disease group (p<0.001). Vanillylmandelic acid was significantly lower in the severe and moderate disease group compared to the control group (p<0.001). Succinic acid was significantly lower in the severe and moderate disease group compared to the control group, in the moderate disease group

Table 1. Comparision of organic acids between groups

Parameters (mmol/molcrea)	Severe Patient Group	Moderate Patient Group	Mild Patient Group	Control Group	*p*
Pyruvic Acid	4.16 (0.32-40)	2.86(0.60-34.4)	2.0(0.31-19.3)	1.99(0-9.94)	0.006 [a]
Lactic acid	6.95(1.14-183)	6.10(1.17-88.0)	3.70(1.04-39.5)	4.28(1.0-56.3)	<0.001 [a,e]
Citric acid	1.0(0-29.8)	4.59(0-76.2)	6.60(0-84.6)	13.4(0-46.4)	<0.001[a,b,c]
-ketoglutaric acid	3.72(0-49.2)	5.13(1.17-62.6)	8.77(0-65.0)	18.7(1.85-42.0)	<0.001 [a,b,e]
Succinic Acid	2.75 (0.63-26.8)	2.65(0.70-15.7)	5.32(0.54-62.3)	8.27(0.64-59.9)	<0.001 [a,b,f]
-hydroxybutyric acid	0 (0-1096)	0(0-2243)	0(0-104)	0(0-0)	<0.001 [a,b,f]
Oxalic acid	1.30 (0-15.7)	1.47(0.14-9.55)	1.40(0-37.0)	1.0(0-25)	0.041 [b]
Homovanillic acid	2.51 (0.03-7.65)	1.74(0.96-3.89)	2.34(0.20-12.8)	7.2(0.55-19.8)	<0.001 [a,b,c]
Vanillylmandelic acid	2.87 (0-9.16)	2.02(0-10.2)	2.76(0.94-16.9)	5.20(0-16.9)	<0.001 [a,b]
2-hydroxybutyric acid	0 (0-99)	0(0-23)	0(0-19.8)	0(0-0)	0.001 [a]

a=significant difference between severe disease control group,
b= significant difference between moderate disease control group,
c= significant difference between mild disease control group
d= significant difference between severe and moderate disease group
e= significant difference between severe and mild disease group
f= significant difference between moderate and mild disease group

compared to the mild disease group (p<0.001). The level of ß-hydroxybutyric acid was significantly higher in the severe and moderate disease group compared to the control group, in the moderate disease group compared to the mild disease group (p<0.001). Oxalic acid was significantly higher in the moderate disease group compared to the control group (p=0.041).

The binary logistic regression model included, α-ketoglutaric acid, succinic acid, and HVA. It was statistically significant with x2 = 98.680; p < 0.001. The model correctly classified 90.5% of the cases. After elimination of the effects of potential confounders namely age, gender, and chronic disease parameters included in the model,increasing α-ketoglutaric acid, succinic acid and HVA were independent predictors of the disease severity with the likelihood ratios shown in Table 2.

Table 2. Odds ratios and coefficients of binary logistic regression analysis of factors associated with disease severity

Parameters	B	p	Exp(B)	95% C.I.for EXP(B)	
-ketoglutaric Acid	0.098	0.009	1.103	1.025	1.186
Succinic Acid	0.129	0.002	1.138	1.049	1.234
Homovanillic Acid	0.323	0.045	1.382	1.008	1.894

DISCUSSION

Due to the nature of the Covid-19 disease, a wide spectrum and different severity of symptoms may present in patients. Currently, co-morbidities such as age, obesity, lung disease, diabetes, and cancer are accepted as risk factors for the severity of the disease. However, unexpected results can be encountered and some of the patients eventually develop progressive disease (10).The mechanisms which cases have complications remain unclear. Identification of spesific metabolites with altered concentration might facilitate the developments of novel therapies and may improve responses to currently available therapies.

In this study, we measured 104 urine organic acid levels by using GC/MS to evaluate carbohydrate metabolism, lipid metabolism, amino acid metabolism and neurotransmitter metabolism in mild, moderate and severe Covid-19 patients. To our knowledge, the first study in which organic acids to include HVA and VMA were measured in Covid-19 patients. We identified that pyruvic and lactic acid concretions were increased whereas TCA cycle intermediates were decreased. Also keton bodies concentrations were high. We evaluated the results according to the severity of the disease and compared them with the control group. The elevation in 2-hydroxybutyric acid may be valuable in demonstrating which patient is at risk of DM.

During glycolysis, 2 molecules of glucose are converted to pyruvate. Pyruvate has several distinct consequences, depending on the microcellular environment, i.e. oxygen availability and energy demand. In the presence of mitochondria and oxygen, pyruvate enters the citric acid cycle and undergoes oxidative phosphorylation. In the absence of mitochondria or oxygen, pyruvate remains in the cytoplasm and is converted to lactate by anaerobic glycolysis by the enzyme LDH. This reaction allows for the regeneration of NAD+, which is necessary for the continuation of glycolysis. 2 ATPs are produced in anaerobic glycolysis, compared to 30 or 32 ATPs produced in oxidative phosphorylation based on the substrate shuttle. Increased blood lactate levels in cases of sepsis are considered as poor prognosis and are associated with increased mortality (11).

For this reason, blood lactate levels were studied in Covid-19 cases with the thought that it would be associated with the disease prognosis, but blood lactate levels were found to be borderline high or normal in some studies. The most important reason for this situation is the deterioration of mitochondrial functions independent of hypoxia in sepsis patients, but the preservation of mitochondrial functions in patients who develop covid pneumonia (12-14). In our study, we found higherpurivic acid levels in the severe patient group than in the other disease groups and control group. We found higher lactic acid levels in the severe disease group compared to the mild disease group and control group (Figure 2). In addition, we found citric acid, one of the citric acid cycle metabolites, to be significantly lower than the control group in all disease groups, the lowest in the severe disease group. On the other hand, α-ketoglutaric acidwas lower in all disease groups compared to the control group, succinic acid levels were lower in the severe and moderate patient group compared to the control group (Figure 3). There was no significant difference in the levels of fumaric acid, malic acid and which are other TCAmetabolites. At the same time, Shi et al. found the blood citric acid level to be low in Covid-19 patients, while they found the succinic acid level to be high(15). L Bo-Wen et al. found TCA enzymes lower in the patient group than in the control group byusing LC-MS/MS (16). The shift of glucose oxidation to anaerobic glycolysis duringhypoxia may be the main cause of the change in TCA metabolites. In addition, TCA cycle intermediate metabolites play a key role in viral replication and virus-induced inflammation (17).

Ketone bodies (ß-hydroxybutyrate, acetoacetate and acetone) are synthesized from fatty acids in the liver and may increase in the blood following a prolonged fasting or ketogenic diet. Ketosis is an important complication of type 1 diabetes pathologically. Therefore, it is important to determine whether it is a physiological adaptation mechanism or whether it develops due to a pathological condition. High blood ketone bodies can also be observed in hospitalized patients, and it is important especially in intensive care patients because it causes acidosis, which is undesirable and needs to be corrected urgently (18). Acidosis is a marker associated with in Covid-19 mortality (19).

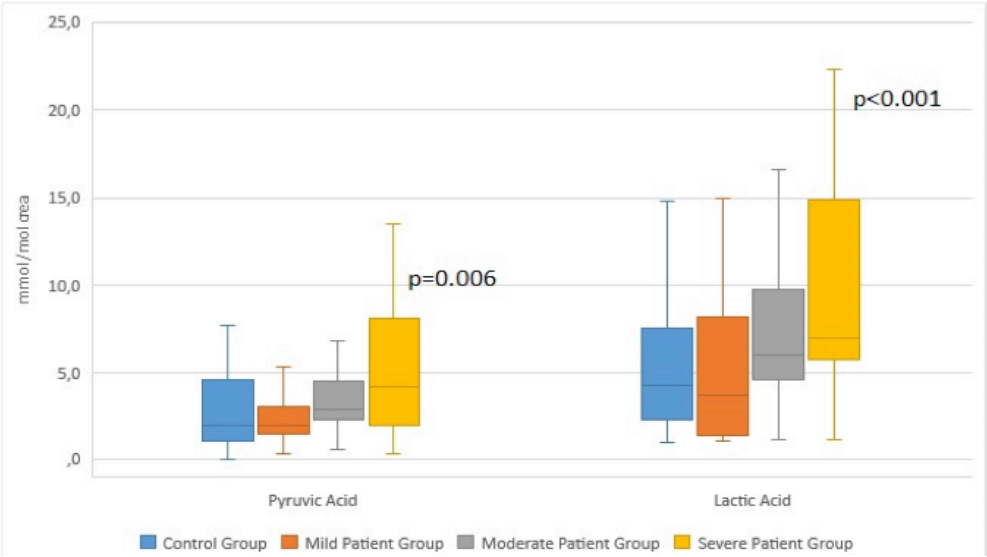

Figure 2. Median Values of Pyruvic Acid and Lactic Acid Median Values in Patient Groups and Control Group

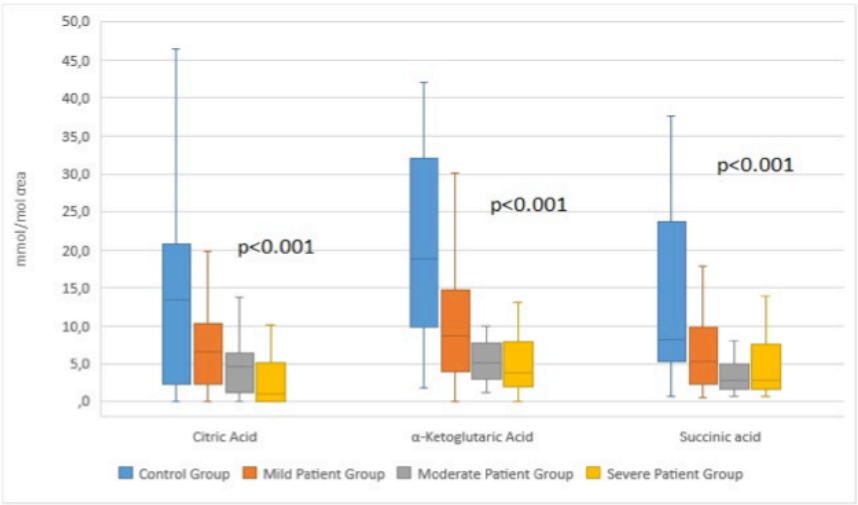

Figure 3. Median Values of Citric Acid, α-Ketoglutaric Acid and Succinic Acid Levels in Patient Groups and Control Group

In our study, we found the level of ß-hydroxybutyric acid to be significantly higher in severe and moderate disease groups compared to the control group. While 46 of 120 covid positive patients had ß-hydroxybutyric excretion in their urine, 28 of these patients did not have a diagnosed diabetes mellitus, Juyi et al found ketone positivity in the blood or urine of 42 patients, 27 of whom did not have diabetes (18).

Oxalic acid is formed mainly due to a diet of plant origin, it can also be formed as a result of metabolism of ascorbic acid, isocitrate and oxoloacetic acid. It is not well metabolized in the human body, most of it is excreted in the urine and increases the risk of kidney stones. It is known that Covid-19 patients spontaneously start using vitamins after diagnosis. Besides, it is stated that IV vitamin C infusion in hospitalized patients reduces mortality and decreases the production of proinflammatory markers, and vitamin C is used as a part of treatment (20, 21). In addition to viral infection, excessive vitamin C intake is also blamed in acute kidney injury that occurs after acute respiratory distress syndrome (ARDS) in Covid 19 positive patients (20). In our study, we found higher oxalic acid levels in all three disease groups compared to the control group. However, there was no relationship between oxalic acid elevation and disease severity. Therefore, we think that higher oxalic acid level may be associated with higher vitamin C intake compared to the control group.

The adrenal glands can be infected by bacteria, viruses, fungi and parasites. Infections caused by these pathogens can cause tissue damage and endocrine dysfunction. In autopsy samples, SARS-CoV-2 has been shown to cause degeneration and necrosis in the adrenal glands. In addition, due to the molecular similarity of autoantibodies formed against some amino acids synthesized by SARS-CoV-2, they can attack the host's own molecules, which may cause endocrine dysfunction such as inadequate corticosteroid response (22, 23). In addition, it is claimed that the endocrine response itself may be related to disease severity. It has been shown that viral infections are milder in diseases and occupations with more epinephrine fluctuation. More fluctuation of epinephrine is shown as a reason for having a milder Covid-19 disease in children (24). In our study, we found that adrenaline and noradrenaline metabolites VMA and HVA were lower in the patient group compared to the control group (Figure 4). These low values were associated with disease severity. We could not find a study in the literature that measured urinary VMA and HVA levels in Covid-19 patients.

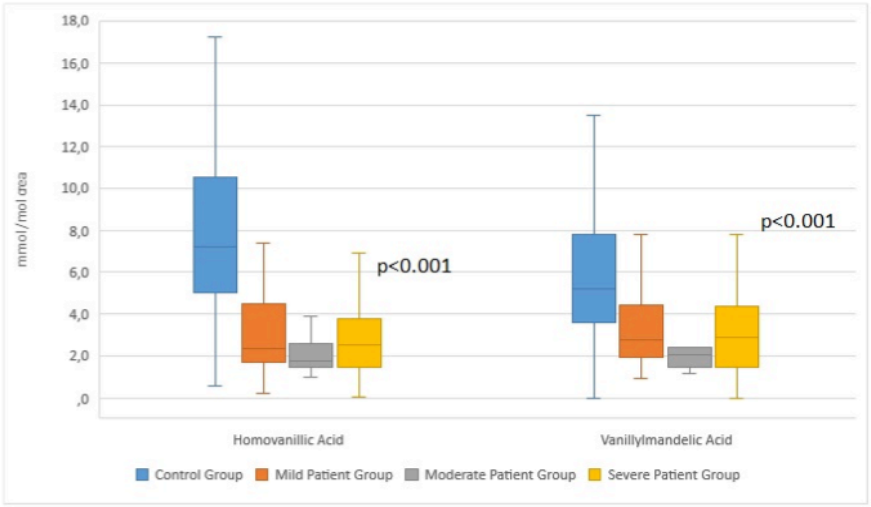

Figure 4. Median Values of Homovanilic Acid and Vanilmandelic Acid in Patient Groups and Control Group

2-Hydroxybutyric Acid or α-hydroxybutyrate is an organic acid originating from α-ketobutyrate, which is metabolized into propionyl-CoA and carbon dioxide formed during the catabolism of sulfur-containing amino acids or glutathione anabolism. 2-Hydroxybutyric Acid is also formed during the synthesis of cysteine from cystathionine. In the case of increased oxidative stress, cystathionine, which is formed as a result of the combination of homocysteine with serine, is hydrolyzed to cysteine, and eventually cysteine is converted to glutathione.For this reason, it is claimed that 2-Hydroxybutyric Acid increases in oxidative stres (25). In addition, it has been argued that the NADH/NAD+ ratio increases as a result of lipid oxidation and is associated with insulin resistance and glucose intolerance in the non-diabetic population (25, 26).Multiple lung disease caused by Sars-COV 2 causes hypoxia to increase reactive oxygen species and oxidative stress. There are many studies in the literature showing that oxidative stress increases in Covid-19 patients (27). In addition, it is stated that Covid-19 damages insulin-secreting beta cells with a direct cytotoxic effect. Studies have shown that there may be moderate pancreatic damage after Covid-19 and that the virus may trigger new onset diabetes (28-31).In our study, we found the level of 2-Hydroxybutyric acid to be higher in the severe disease group compared to the control group. We think that this elevation may be due to increased oxidative stress. However, we do not know whether this elevation increases secondary to the damage to the pancreas. Therefore, we think that these data should be confirmed by experimental studies.

The empirical results reported herein should be considered in the light of some limitations. We used spot urine samples by proportioning organic acid results to creatinine. Despite this, 24h urine collection may be more accurate in VMA and HVA tests in order to eliminate diurnal variation. In the present study, although we tried to ensure that there was no difference between the ages of the groups, there were more elderly individuals in the severe and moderate disease groups due to Covid-19 disease nature. To minimise this age gap along with the other potential confounders, namely gender, and chronic disease parameters were included in the model to define independent predictors of disease severity.

Overall, it may be said organic acid measurement can be considered in Covid-19 patients especially for follow-up and management of the disease. Due to α-ketoglutaric acid, succinic acid and HVA were independent predictors of the disease severity, measurement of these spesific metabolites might facilitate the developments of novel therapies and may improve responses to currently available therapies with the ease of prognosis prediction in Covid-19 patients.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Ankara City Hospital Clinic Researches Ethics Committee (Date: 03.11.2021 and Number: E1-21-2110).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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