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ORIGINAL ARTICLE

Investigation of Urine Organic Acid Profile in Coronavirus Disease (COVID-19) Patients

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SUMMARY

Backgrounds: **Coronavirus disease 2019 (COVID-19) is a viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The high mortality and morbidity rate and its course with a wide variety of clinical symptoms indicate that the disease affects many metabolic pathways. The aim of our study was to investigate the metabolic effects of SARS-CoV-2 by evaluating the urinary organic acid profile in patients with SARS-CoV-2 infection. Can metabolites in urine guide the diagnosis, follow-up, treatment, and prognosis of COVID-19?**
Methods: **Forty-two patients, including 30 SARS-CoV-2 RT-PCR positive patients and 12 SARS-CoV-2 RT-PCR negative controls, were studied. SARS-COV-2 RT-PCR of nasopharyngeal swab samples was studied. Urines were evaluated in the GCMS-QP2010 SE Gas Chromatograph Mass Spectrometer (SHIMADZU) device for organic acid profile in the metabolism laboratory. Urine organic acid profile was evaluated by studying 117 organic acids in each patient.**
Results: **Tiglylglycine, 2-hydroxybutyric acid, 4-hydroxyphenylpyruvic acid, 3-hydroxypropionic acid, erythro-4,5-dihydroxyhexanoic acid lactone, 2-hydroxyphenylacetic acid, N-acetyltyrosine, 3-phenyllactic acid, 5-hydroxyindolacetic acid, 3-hydroxysebacic acid, palmitic acid, 3-methylglutaconic acid, 3-methylglutaric acid, lactic acid, pyruvic acid-oxime, 3-hydroxysobutyric acid, and organic acids were found to be increased, compared to the control group.**
Conclusions: **Tiglylglycine has been specifically identified as a potential biomarker of respiratory chain disorders. The deterioration in lipid metabolism and pyruvate pathway in COVID-19 patients was evaluated as remarkable.**
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COVID-19, SARS-CoV-2, urine organic acid

INTRODUCTION

The COVID-19 pandemic, caused by the acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) from the coronavirus family, first appeared in the People's Republic of China in 2019. COVID-19 affected all countries and was declared a pandemic by the World Health Organization [1]. SARS-CoV-2 is a positive-stranded RNA betacoronavirus. SARS-CoV-2 has tropism for

various tissues, including the respiratory tract, brain, endothelium, heart, kidney, and liver [2]. During the course of the infection, abnormally high immunological response, serious symptoms, and consequences are observed [3]. In laboratory parameters, high CRP, low lymphocyte, high LDH, and elevated D-dimer are detected in serum as bad prognostic markers [4]. Since the beginning of the pandemic, studies have been carried out to detect viruses. The World Health Organization recommended identifying the disease with RT-PCR [5]. Antigen-antibody tests and lung tomography findings have been instructive. Studies aiming to make a faster diagnosis, such as the urine foam test developed in our country, have been conducted [6]. Urine biomarkers reflecting metabolic abnormalities in COVID-19 patients have been found in previous research [7]. Since GC/MS gives us a wide spectrum of the metabolites present in urine, we have employed this approach to better define these metabolic changes in COVID-19. The aim of our study was to investigate the metabolic effects of SARS-CoV-2 by evaluating the urinary organic acid profile in patients with SARS-CoV-2 infection. We examined whether there is a metabolite in urine that has predictive value in the diagnosis, follow-up, treatment, and prognosis of COVID-19.

MATERIALS AND METHODS

Sample collection

In the power analysis performed with the G*power 3.1 program and related to our study, the effect size for Glycine between the control and study groups was found to be 0.71 [7] (alpha error probability = 0.05); the total number of samples to be taken in the sample size analysis performed by taking the power value 0.80 was found to be 40. The study included 42 subjects, 30 patients and 12 healthy controls. Urines of 30 hospitalized patients, who were found to have a positive SARS-CoV-2 RT-PCR test taken in the infection clinic of our hospital, were taken and evaluated in the laboratory. Adult and inpatient groups were included in the study. Pediatric patients, intensive care patients, and outpatients were excluded from the study. In the control group, people who were positive or suspected of SARS-CoV-2, people who recently had COVID 19, and people with any metabolic disease were excluded. Consent forms were filled and signed by the patients. SARS-COV-2 RT-PCR of nasopharyngeal swab samples was studied. Urines were evaluated in the GCMS-QP2010 SE Gas Chromatograph Mass Spectrometer (SHIMADZU) device for organic acid profile in the metabolism laboratory. Urine organic acid profile was evaluated by studying 117 organic acids in each patient. Organic acid levels were evaluated statistically in the patient group with normal creatinine levels by comparing them with the reference range and control group. It was a prospective observational study.

RT-PCR protocol

Nasopharyngeal swab samples of patients, the SARS-CoV-2 detection method, was performed by the SARS-CoV-2 qPCR test of Direct Detection SARS-CoV-2 qPCR (RTA kit Ltd.) on BioRad CFX 96 platform (California, USA), according to the protocols provided by the manufacturer.

Urine organic acid procedure

In this method, 4-phenylbutyric acid was used as internal standard. For reliable identification of ketoacids with hydroxylamine, oxidation was performed. The pH of the urine was made acidic with hydrochloric acid and was incubated. The samples were saturated with NaCl. Ethyl acetate was used as the carrier phase. Evaporation was done by separating the organic phase with ethyl acetate. After the evaporation process, the samples were dissolved with ethyl acetate, and anhydrous NaSO4 was added to the dehydration process. After centrifugation, the supernatant was taken into clean tubes and the second evaporation was performed. The samples were derivatized with trimethylsilyl derivative at 65°C. After the derivatization process, the insert vial was taken and the injection was made in the GCMS device. Urine creatinine value was measured for quantitative result.

Statistical analysis

NCSS (Number cruncher statistical system) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, and maximum) were used while evaluating the study data. The conformity of the quantitative data to the normal distribution was tested with the Shapiro-Wilk test and graphical examinations. The Mann-Whitney U test was used for comparisons between two groups of quantitative variables that did not show normal distribution. Statistical significance was accepted as $p < 0.05$.

RESULTS

The study was conducted in the Kanuni Sultan Suleyman Research and Training Hospital with a total of 42 patients, with 71.4% (n = 30) in the SARS-CoV-2 group and 28.6% (n = 12) in the control group. The mean age was 40 ± 5 years. Result differences occurred between normal and SARS-CoV-2 patients during the organic acid procedure. A thick layer of pellets was formed in SARS-CoV-2 patients during the centrifugation stage during the organic acid pretreatment of the patients (Figure 1). In the later phase, which is the evaporation stage, dense material adhered to the tube wall was detected in SARS-CoV-2 patients, compared to normal urine (Figure 2). The increasing organic acids in the urine and the odds ratio of how many times it increased compared to the normal patient and reference values are presented in Table 1. The organic acids decreased in urine are given in Table 2. The group with no change in

Table 1. Increased organic acids in urine in the SARS-CoV-2-positive patient group.

		Control (n = 12)	SARS-CoV-2 (n = 30)	Total	p	OR 95% CI
Lactic acid	median ± Sd	2.76 ± 2.52	9.06 ± 13.69	7.26 ± 11.94	[a] 0.015 *	1.304
	mean (min-max)	2.06 (0.5 - 9)	5.36 (1.1 - 71)	3.61 (0.5 - 71)		0.986 - 1.724
2-Hydroxybutyric acid	median ± Sd	0 ± 0	0.85 ± 1.65	0.6 ± 1.44	[a] 0.036 *	-
	mean (min-max)	0 (0 - 0)	0 (0 - 5.8)	0 (0 - 5.8)		
3-Hydroxypropionic acid	median ± Sd	0.30 ± 0.25	0.63 ± 0.45	0.53 ± 0.43	[a] 0.017 *	24.688
	mean (min-max)	0.27 (0 - 0.8)	0.5 (0 - 2.2)	0.46 (0 - 2.2)		1.41 - 432.33
Pyruvic acid-oxime	median ± Sd	5.58 ± 3.21	6.46 ± 3.34	6.21 ± 3.29	[a] 0.419	1.093
	mean (min-max)	5.31 (1.8 - 12.4)	5.71 (2 - 15.2)	5.69 (1.8 - 15.2)		0.875 - 1.364
3-Hydroxyisobutyric acid	median ± Sd	2.15 ± 3.15	3.99 ± 3.43	3.46 ± 3.42	[a] 0.049 *	1.209
	mean (min-max)	0.92 (0 - 9.5)	3.6 (0 - 11.5)	2.57 (0 - 11.5)		0.947 - 1.544
2-Hydroxyisovaleric acid	median ± Sd	0 ± 0	0.34 ± 0.67	0.25 ± 0.58	[a] 0.036 *	-
	mean (min-max)	0 (0 - 0)	0 (0 - 2.4)	0 (0 - 2.4)		
Erythro-4,5-dihydroxyhexanoic acid lactone	median ± Sd	0.08 ± 0.19	0.28 ± 0.34	0.23 ± 0.32	[a] 0.012 *	22.539
	mean (min-max)	0 (0 - 0.5)	0.15 (0 - 1.3)	0.07 (0 - 1.3)		0.57 - 885.188
3-Methylglutaconic acid	median ± Sd	0.86 ± 0.71	1.86 ± 1.14	1.57 ± 1.13	[a] 0.010 *	2.854
	mean (min-max)	0.77 (0 - 2.3)	1.94 (0 - 4.7)	1.51 (0 - 4.7)		1.231 - 6.621
3-Methylglutaric acid	Median ± Sd	0.34 ± 0.31	0.4 ± 0.3	0.38 ± 0.3	[a] 0.460	2.086
	mean (min-max)	0.31 (0 - 1)	0.31 (0 - 1.1)	0.31 (0 - 1.1)		0.187 - 23.258
Tiglylglycine	median ± Sd	0 ± 0	1.06 ± 1.19	0.76 ± 1.11	[a] 0.001 **	-
	mean (min-max)	0 (0 - 0)	0.69 (0 - 4.4)	0.1 (0 - 4.4)		
2-Hydroxyphenylacetic acid	median ± Sd	0.19 ± 0.19	0.56 ± 0.6	0.46 ± 0.54	[a] 0.049 *	10.247
	mean (min-max)	0.17 (0 - 0.5)	0.51 (0 - 2.5)	0.33 (0 - 2.5)		0.992 - 105.89
3-Phenyllactic acid	median ± Sd	0 ± 0	1.47 ± 6.3	1.05 ± 5.34	[a] 0.001 **	-
	mean (min-max)	0 (0 - 0)	0.17 (0 - 34.7)	0 (0 - 34.7)		
4-Hydroxyphenylpyruvic acid	median ± Sd	0.09 ± 0.23	2.7 ± 7.54	1.96 ± 6.45	[a] 0.001 **	72.418
	mean (min-max)	0 (0 - 0.8)	0.86 (0 - 41.9)	0.6 (0 - 41.9)		3.18 - 1,647.24
Palmitic acid	median ± Sd	1.12 ± 1.17	4.61 ± 3.42	3.61 ± 3.35	[a] 0.001 **	3.536
	mean (min-max)	0.91 (0 - 3.2)	3.9 (1.3 - 17.1)	2.84 (0 - 17.1)		1.452 - 8.613
3-Hydroxysebacic acid	median ± Sd	0.21 ± 0.22	1.17 ± 1.76	0.9 ± 1.55	[a] 0.014 *	9.585
	mean (min-max)	0.14 (0 - 0.6)	0.68 (0 - 9.2)	0.52 (0 - 9.2)		1.34 - 68.71
N-Acetyltyrosine	median ± Sd	0.1 ± 0.2	0.4 ± 0.74	0.32 ± 0.64	[a] 0.060	10.900
	mean (min-max)	0 (0 - 0.7)	0.15 (0 - 3.8)	0 (0 - 3.8)		0.46 - 256.77
5-Hydroxyindolacetic acid	median ± Sd	0.1 ± 0.34	0.78 ± 0.94	0.59 ± 0.87	[a] 0.008 **	7.984
	mean (min-max)	0 (0 - 1.2)	0.66 (0 - 3.9)	0 (0 - 3.9)		1.088 - 58.573

[a] Mann-Whitney U test, * $p < 0.05$, ** $p < 0.01$. (Sd - standard deviation, min - minimum, max - maximum).

Table 2. Decreased organic acids in urine in the SARS-Cov-2 patient group.

		Control (n = 12)	SARS-CoV-2 (n = 30)	Total	p	OR 95% CI
2-Hydroxyisobutyric acid	median ± Sd	2.86 ± 1.45	1.61 ± 1.11	1.97 ± 1.33	[a] 0.018 *	2.239
	mean (min-max)	2.62 (0.9 - 5)	1.67 (0 - 3.6)	1.76 (0 - 5)		1.197 - 4.188
Glycolic acid	median ± Sd	1.62 ± 1.07	0.20 ± 0.84	0.60 ± 1.11	[a] 0.001 **	4.294
	mean (min-max)	1.46 (0-3.5)	0 (0 - 4.4)	0 (0 - 4.4)		1.581 - 11.660
Methylmalonic acid	median ± Sd	0.65 ± 0.82	0.26 ± 0.49	0.37 ± 0.62	[a] 0.041 *	2.628
	mean (min-max)	0.3 (0 - 2.4)	0 (0 - 1.9)	0 (0 - 2.4)		0.881 - 7.842
Benzoic acid	median ± Sd	0.12 ± 0.20	0.69 ± 1.45	0.53 ± 1.25	[a] 0.043 *	11.005
	mean (min-max)	0 (0 - 0.7)	0.21 (0 - 6.6)	0.13 (0 - 6.6)		0.46 - 264.08
Methylsuccinic acid	median ± Sd	1.62 ± 1.24	0.9 ± 0.73	1.11 ± 0.95	[a] 0.016 *	2.300
	mean (min-max)	1.42 (0 - 4.8)	0.78 (0 - 4.3)	0.82 (0 - 4.8)		0.955 - 5.538
Glutaric acid	median ± Sd	0.78 ± 1.26	0.03 ± 0.09	0.25 ± 0.74	[a] 0.001 **	1324.955
	mean (min-max)	0.33 (0 - 4.1)	0 (0 - 0.5)	0 (0 - 4.1)		4.14 - 423,790.6
2-Hydroxyglutaric acid	median ± Sd	0.64 ± 0.49	0.09 ± 0.24	0.24 ± 0.41	[a] 0.001 **	44.783
	mean (min-max)	0.64 (0 - 1.5)	0 (0 - 1)	0 (0 - 1.5)		4.367 - 459.24
3-Hydroxyphenylacetic acid	median ± Sd	3.67 ± 2.03	2.00 ± 2.54	2.48 ± 2.5	[a] 0.009 **	1.296
	mean (min-max)	4.11 (0.2 - 6.4)	0.99 (0 - 10.2)	1.31 (0 - 10.2)		0.985 - 1.704
2-Ketoglutaric acid	median ± Sd	17.86 ±	3.9 ± 1.77	7.89 ± 12.13	[a] 0.001 **	1.785
	mean (min-max)	8.61 (2.8 - 72.9)	3.84 (1 - 8)	4.42 (1 - 72.9)		1.104 - 2.885
N-Acetylaspartic acid	median ± Sd	0.13 ± 0.19	0 ± 0	0.04 ± 0.12	[a] 0.001 **	-
	mean (min-max)	0.04 (0 - 0.6)	0 (0 - 0)	0 (0 - 0.6)		
Homovanillic acid	median ± Sd	5.17 ± 4.12	2.36 ± 1.85	3.17 ± 2.94	[a] 0.021 *	1.400
	mean (min-max)	4.36 (0 - 12.9)	1.8 (0.7 - 10.7)	2.25 (0 - 12.9)		1.055 - 1.859
Citric acid	median ± Sd	7.45 ± 8.82	1.12 ± 2.04	2.93 ± 5.67	[a] 0.006 **	1.378
	mean (min-max)	4.57 (0 - 27.3)	0 (0 - 8.5)	0.33 (0 - 27.3)		1.046 - 1.816
3,4-Dihydroxyphenylacetic acid	median ± Sd	1.00 ± 1.24	0.29 ± 0.59	0.49 ± 0.88	[a] 0.005 **	2.590
	mean (min-max)	0.51 (0 - 4)	0 (0 - 3)	0.07 (0 - 4)		0.988 - 6.792
Succinic semialdehyde-oxime	median ± Sd	0.01 ± 0.02	0.0 ± 0.02	0.01 ± 0.02	[a] 0.010 *	-
	mean (min-max)	0 (0 - 0)	0 (0 - 0.1)	0 (0 - 0.1)		
2-Ethyl-3-hydroxypropionic acid	median ± Sd	1.28 ± 1.62	0.42 ± 0.88	0.67 ± 1.19	[a] 0.002 **	1.799
	mean (min-max)	0.69 (0 - 4.5)	0 (0 - 2.8)	0 (0 - 4.5)		0.995 - 3.254

[a] Mann-Whitney U test, * $p < 0.05$, ** $p < 0.01$. Sd - standard deviation.

Table 3. Organic acids with no significant change in SARS-CoV-2 positive patients compared to normal patients.

		Control (n = 12)	SARS-CoV-2 (n = 30)	Total	p	OR 95% CI
Oxalic acid	median ± Sd	1.88 ± 1.42	1.72 ± 1.66	1.76 ± 1.58	[a] 0.593	1.070
	mean (min-max)	1.77 (0 - 4)	1.41 (0 - 5.6)	1.52 (0 - 5.6)		0.699 - 1.637
3-Hydroxybutyric acid	median ± Sd	2.10 ± 3.00	1.08 ± 3.12	1.37 ± 3.09	[a] 0.068	1.105
	mean (min-max)	0 (0 - 8.6)	0 (0 - 13.4)	0 (0 - 13.4)		0.900 - 1.356
2-Methyl-3-hydroxybutyric acid	median ± Sd	1.13 ± 1.27	0.66 ± 0.88	0.79 ± 1.01	[a] 0.127	1.542
	mean (min-max)	0.56 (0 - 4.4)	0 (0 - 3.1)	0.49 (0 - 4.4)		0.802 - 2.963
3-Hydroxyisovaleric acid	median ± Sd	6.03 ± 6.27	3.90 ± 2.28	4.51 ± 3.9	[a] 0.717	1.145
	mean (min-max)	2.87 (0.6 - 21.1)	3.76 (0.5 - 8.7)	3.54 (0.5 - 21.1)		0.955 - 1.374
4-Hydroxybutyric acid	median ± Sd	0 ± 0	0.19 ± 1.02	0.13 ± 0.86	[a] 0.527	-
	mean (min-max)	0 (0 - 0)	0 (0 - 5.6)	0 (0 - 5.6)		
2-Hydroxyisocaproic acid	median ± Sd	0 ± 0	0.02 ± 0.09	0.01 ± 0.08	[a] 0.262	-
	mean (min-max)	0 (0 - 0)	0 (0 - 0.5)	0 (0 - 0.5)		
Acetoacetic acid (2TMS)	median ± Sd	0.01 ± 0.02	0.15 ± 0.58	0.11 ± 0.5	[a] 0.621	-
	mean (min-max)	0 (0 - 0.1)	0 (0 - 3)	0 (0-3)		
2-Hydroxy-3-methylvaleric acid	median ± Sd	0 ± 0	0 ± 0.01	0 ± 0.01	[a] 0.527	-
	mean (min-max)	0 (0 - 0)	0 (0 - 0.1)	0 (0 - 0.1)		
Acetoacetate	median ± Sd	0.05 ± 0.19	0.05 ± 0.19	0.05 ± 0.19	[a] 0.852	0.821
	mean (min-max)	0 (0 - 0.7)	0 (0 - 1)	0 (0 - 1)		0.026 - 26.286
Octonoic acid	median ± Sd	0 ± 0	0.01 ± 0.03	0 ± 0.03	[a] 0.527	-
	mean (min-max)	0 (0 - 0)	0 (0 - 0.2)	0 (0 - 0.2)		
Glycerol	median ± Sd	0.25 ± 0.47	0.17 ± 0.52	0.19 ± 0.5	[a] 0.137	1.345
	mean (min-max)	0.16 (0 - 1.7)	0 (0 - 2.8)	0.01 (0 - 2.8)		0.388 - 4.660
Ethylmalonic acid	median ± Sd	1.98 ± 1.87	1.33 ± 1.65	1.51 ± 1.72	[a] 0.165	1.232
	mean (min-max)	1.27 (0 - 6.4)	0.95 (0 - 7.5)	1.03 (0 - 7.5)		0.846 - 1.793
2-Ketoisocaproic acid	median ± Sd	0.71 ± 0.45	0.53 ± 0.57	0.58 ± 0.54	[a] 0.183	1.841
	mean (min-max)	0.78 (0 - 1.5)	0.34 (0 - 2)	0.41 (0-2)		0.540 - 6.280
Phenylacetic acid	median ± Sd	0 ± 0	0.01 ± 0.05	0.01 ± 0.05	[a] 0.365	-
	mean (min-max)	0 (0 - 0)	0 (0 - 0.3)	0 (0 - 0.3)		
Succinic acid	median ± Sd	1.75 ± 1.28	1.26 ± 1.38	1.4 ± 1.36	[a] 0.172	1.279
	mean (min-max)	1.63 (0.3 - 4.6)	0.79 (0 - 6.1)	0.96 (0 - 6.1)		0.797 - 2.053
Glyceric acid	median ± Sd	0 ± 0.01	0.01 ± 0.02	0 ± 0.02	[a] 0.901	-
	mean (min-max)	0 (0 - 0)	0 (0 - 0.1)	0 (0 - 0.1)		
Fumaric acid	median ± Sd	0.23 ± 0.44	0.25 ± 0.3	0.24 ± 0.34	[a] 0.620	1.165
	mean (min-max)	0 (0 - 1.4)	0.08 (0-1)	0 (0 - 1.4)		0.157 - 8.641
Uracil	median ± Sd	0.06 ± 0.19	0.04 ± 0.11	0.04 ± 0.14	[a] 0.553	3.753
	mean (min-max)	0 (0 - 0.7)	0 (0-0.5)	0 (0 - 0.7)		0.038 - 366.265
Threo-4,5-dihydroxyhexanoic lactone	median ± Sd	0.17 ± 0.43	0.11 ± 0.24	0.13 ± 0.3	[a] 0.971	1.774
	mean (min-max)	0 (0 - 1.4)	0 (0-0.9)	0 (0 - 1.4)		0.218 - 14.425
2-Hydroxyglutaric acid lacton	median ± Sd	0.04 ± 0.13	0.03 ± 0.12	0.03 ± 0.12	[a] 0.891	1.293
	mean (min-max)	0 (0 - 0.4)	0 (0 - 0.6)	0 (0 - 0.6)		0.006 - 281.181

Table 3. Organic acids with no significant change in SARS-CoV-2 positive patients compared to normal patients (continued).

		Control (n = 12)	SARS-CoV-2 (n = 30)	Total	p	OR 95% CI
Isobutyrylglycine	median ± Sd	0.07 ± 0.24	0.11 ± 0.17	0.1 ± 0.19	[a] 0.112	3.948
	mean (min-max)	0 (0 - 0.8)	0 (0 - 0.7)	0 (0 - 0.8)		0.061 - 253.57
Isovalerylglycine	median ± Sd	0.3 ± 0.78	0.07 ± 0.15	0.14 ± 0.44	[a] 0.641	3.890
	mean (min-max)	0 (0 - 2.7)	0 (0 - 0.5)	0 (0 - 2.7)		0.350 - 43.239
Malic acid	median ± Sd	0 ± 0	0.02 ± 0.08	0.01 ± 0.07	[a] 0.365	-
	mean (min-max)	0 (0 - 0)	0 (0 - 0.4)	0 (0 - 0.4)		
Adipic acid	median ± Sd	1.39 ± 0.83	1.40 ± 1.30	1.40 ± 1.17	[a] 0.867	1.003
	mean (min-max)	1.12 (0.3 - 2.8)	1.25 (0 - 7)	1.24 (0 - 7)		0.563 - 1.790
5-Oxoproline	median ± Sd	0 ± 0	0.04 ± 0.24	0.03 ± 0.2	[a] 0.527	-
	mean (min-max)	0 (0 - 0)	0 (0 - 1.3)	0 (0 - 1.3)		
3-Methyladipic acid	median ± Sd	1.29 ± 1.46	0.92 ± 1.22	1.03 ± 1.29	[a] 0.212	1.246
	mean (min-max)	0.85 (0 - 4.6)	0 (0 - 3.3)	0.55 (0 - 4.6)		0.750 - 2.070
3-Hydroxy-3-methylglutaric acid	median ± Sd	1.19 ± 0.82	0.97 ± 0.36	1.03 ± 0.53	[a] 0.749	2.128
	mean (min-max)	1 (0 - 2.4)	0.95 (0 - 1.7)	0.95 (0 - 2.4)		0.597 - 7.583
Hexanoylglycine	median ± Sd	0 ± 0	0.04 ± 0.18	0.03 ± 0.15	[a] 0.262	-
	mean (min-max)	0 (0 - 0)	0 (0 - 1)	0 (0-1)		
4-Hydroxyphenylacetic acid	median ± Sd	19.39 ± 14.23	22.12 ± 16.51	21.34 ± 15.77	[a] 0.559	1.012
	mean (min-max)	16.04 (5 - 52.4)	16.26 (6 - 80.2)	16.14 (5 - 80.2)		0.966 - 1.061
Aconitic acid	median ± Sd	4.73 ± 4.15	3.02 ± 2.96	3.51 ± 3.38	[a] 0.278	1.161
	mean (min-max)	4.63 (0 - 14.9)	1.99 (0 - 10.3)	4.04 (0 - 14.9)		0.946 - 1.423
Orotic acid	median ± Sd	0.73 ± 2.51	0 ± 0	0.21 ± 1.34	[a] 0.114	-
	mean (min-max)	0 (0 - 8.7)	0 (0 - 0)	0 (0 - 8.7)		
Hippuric acid	median ± Sd	136.63 ± 178.93	48.41 ± 45.21	73.62 ± 107.99	[a] 0.316	1.009
	mean (min-max)	56.4 (0 - 582.1)	37.44 (0 - 182.1)	43.19 (0 - 582.1)		1.000 - 1.018
Homogentisic acid	median ± Sd	0 ± 0	0.14 ± 0.59	0.1 ± 0.5	[a] 0.365	-
	mean (min-max)	0 (0 - 0)	0 (0 - 3)	0 (0 - 3)		
Vanilmandelic acid	median ± Sd	2.67 ± 2.39	2.82 ± 1.74	2.77 ± 1.92	[a] 0.316	1.042
	mean (min-max)	1.93 (0 - 8.1)	2.29 (0 - 9.4)	2.2 (0 - 9.4)		0.725 - 1.497
3-Indolasetic acid	median ± Sd	0.04 ± 0.15	0.15 ± 0.4	0.12 ± 0.35	[a] 0.586	3.367
	mean (min-max)	0 (0 - 0.5)	0 (0 - 1.7)	0 (0 - 1.7)		0.173 - 65.51
3-Hydroxyhippuric acid	median ± Sd	25.54 ± 75.32	1.79 ± 2.8	8.57 ± 40.56	[a] 0.131	1.181
	mean (min-max)	3.15 (0 - 264.3)	0.52 (0 - 12)	0.81 (0 - 264.3)		0.967 - 1.443
Stearic acid	median ± Sd	0.2 ± 0.69	0.54 ± 1.57	0.44 ± 1.38	[a] 0.478	1.277
	mean (min-max)	0 (0 - 2.4)	0 (0 - 6.6)	0 (0 - 6.6)		0.636 - 2.564
2-Methylbutrylglycine	median ± Sd	0.04 ± 0.13	0.04 ± 0.11	0.04 ± 0.12	[a] 1.000	0.808
	mean (min-max)	0 (0 - 0.4)	0 (0 - 0.6)	0 (0-0.6)		0.003 - 247.28
Octenedioic acid	median ± Sd	1.14 ± 0.7	1.95 ± 1.25	1.72 ± 1.17	[a] 0.112	2.440
	mean (min-max)	1.31 (0 - 2)	1.4 (0.7 - 4.9)	1.37 (0 - 4.9)		0.964 - 6.178
Azelaic acid	median ± Sd	2.62 ± 2.87	1.4 ± 1.08	1.75 ± 1.83	[a] 0.451	1.436
	mean (min-max)	1.41 (0 - 8.8)	1.27 (0 - 3.4)	1.28 (0 - 8.8)		0.962 - 2.144

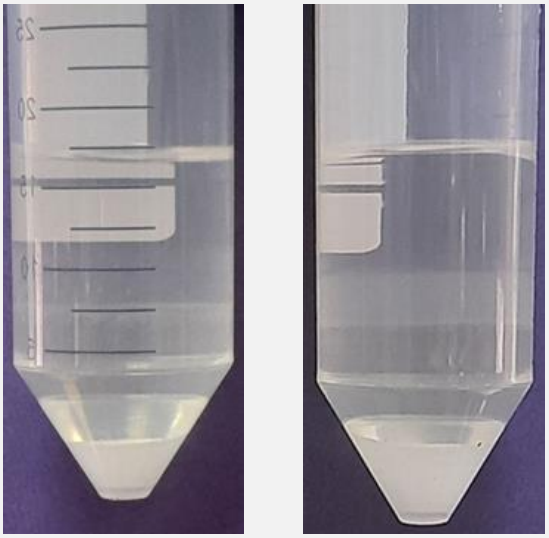

Figure 1. Images during the centrifugation stage of the urine organic acid pretreatments.

Left: SARS-COV-2 negative; right: SARS-Cov-2 positive.

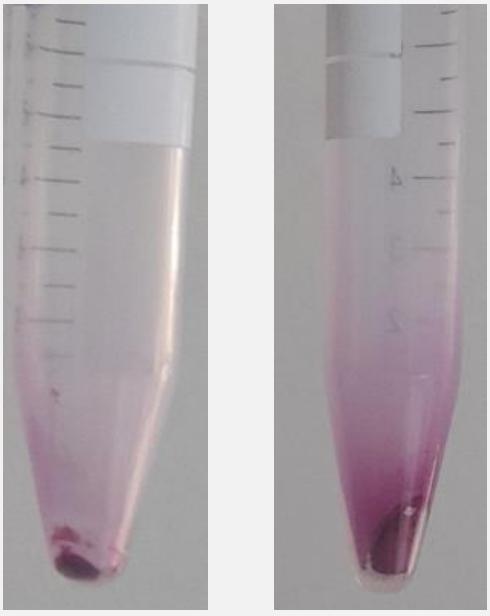

Figure 2. Evaporation phase images after pelleting after the centrifugation step of the urine organic acid pretreatments.

Left: SARS-COV-2 negative; right: SARS-Cov-2 positive.

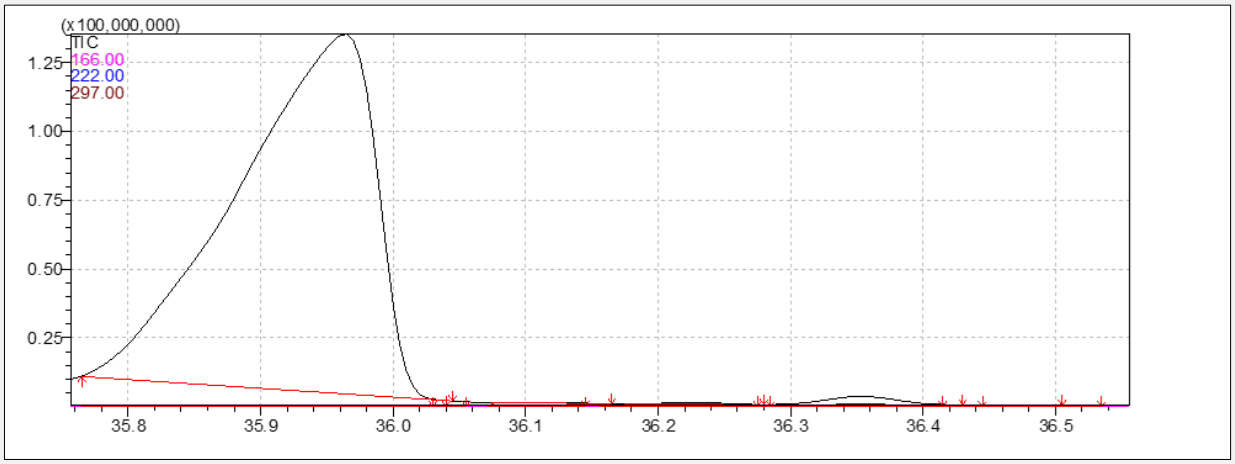

Figure 3. Peak seen in the urine of all SARS-CoV-2 patients, rising between 35.8 - 36 molecular ion (m/e), but not detectable in normal urine, which does not match the data in the device library.

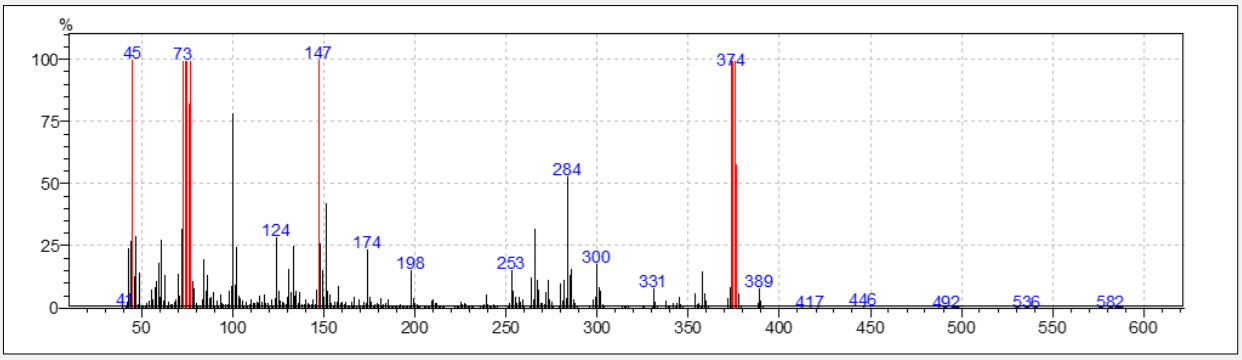

Figure 4. The ions of the metabolite, the peak of which is seen in Figure 3.

urine, according to the normal patient group and reference values, is presented in Table 3. According to the device library, an unidentified peak was detected and the ions belonging to this peak are given in Figure 3 and Figure 4.

DISCUSSION

In this study, in the SARS-CoV-2-positive patient group, tiglylglycine, 2-hydroxybutyric acid, 4-hydroxyphenylpyruvic acid, 3-hydroxypropionic acid, erythro-4,5-dihydroxyhexanoic acid lactone, 2-hydroxyphenylacetic acid, N-acetyltyrosine, 3-phenyllactic acid, 5-hydroxyindolacetic acid, 3-hydroxysebacic acid, palmitic acid, 3-methylglutaconic acid, 3-methylglutaric acid, lactic acid, pyruvic acid-oxime, and 3-hydroxyisobutyric acid were found to increase. Specifically, tiglylglycine is an acyl glycine. Acylglycines are normally minor metabolites of fatty acids. It is an intermediate in isoleucine catabolism, it is elevated in the urine in pa-

tients with beta-ketothiolase deficiency or disorders of propionate metabolism, and it is the first urinary compound shown as a useful biomarker in respiratory chain disorders [8,9]. Higher levels of tiglylglycine are a significant indicator in the COVID-19 patient group.

5-hydroxyindolacetic acid is the primary metabolite of serotonin, a hormone derived from the amino acid trypotphan. Serotonin is stored in enterochromaffin (EC) cells in the gastrointestinal tract (GIS) mucosa, serotonergic neurons in the brain, pineal gland, and platelets. The amount of 5-hydroxyindoleacetic acid (5-HIAA) in the urine is a measure of serotonin synthesis and degradation. Serotonin plays a role in platelet regulation and homeostasis [10,11]. There is an increase in the urine levels in COVID-19 patients. It has been thought that COVID-19 may also be associated with platelet levels. The increase in 2-hydroxyphenylacetic acid, pyruvic acid-oxime, 3-phenyllactic acid, lactic acid, and 4-hydroxyphenylpyruvic acid is an indication that it plays a role in pyruvate metabolism and that there is a problem in the pyruvate pathway. Elevated LDH levels are an indicator in SARS-CoV-2 patients. LDH is an intracellular enzyme that catalyzes pyruvate and facilitates glycolysis. It is released into the blood after cell death. SARS-CoV-2 diabetes and cancer are also elevated. It is associated with mortality [12]. COVID-19 is also associated with mortality and ARDS [13].

The 3-hydroxysobutyric acid is produced in the liver in mammalian tissues that catabolize 2-hydroxybutyric acid and L-threonine and synthesize glutathione. Hepatic glutathione synthesis increases significantly under oxidative stress and metabolic stress conditions. Under such metabolic stress conditions, sources of L-cysteine for glutathione synthesis become limited, so homocysteine is diverted from the methionine-forming transmethylation pathway to the cystathionine-forming transsulfuration pathway, and 2-hydroxybutyric acid is released as a byproduct when cystathionine occurs. It appears as an early indicator of insulin resistance as well as worsening glucose tolerance [14]. The increase in the urine level in COVID-19 patients is determined as a biomarker for metabolic stress and the emergence of irregular blood sugar in patients.

Increase in 3-hydroxysebacic acid, palmitic acid, 3-methylglutaconic acid, 3-methylglutaric acid, and 5-hydroxyindolacetic acid is related to fat metabolism, and studies have been conducted on especially high palmitic acid in SARS-Cov-2 patients [15].

In our study, an increase was detected in the molecules used in the synthesis of fatty acid in urine. The excretion of 3-hydroxysebacic acid in urine, especially during medium and long chain triglyceride diets, indicates a problem in fat metabolism [16]. It is an indication that lipid metabolism disorders occur during the course of the disease [17].

N-acetyltyrosine belongs to the class of organic compounds known as tyrosine and its derivatives. Tyrosine and its derivatives are compounds containing tyrosine or a derivative thereof, resulting from the reaction of tyrosine at the amino group or carboxy group, or the replacement of any hydrogen of glycine with a heteroatom. N-acetyltyrosine is a secondary metabolite. Secondary metabolites are metabolically or physiologically nonessential metabolites that can act as defense or signaling molecules. In some cases, these are simple molecules resulting from incomplete metabolism of other secondary metabolites. Few articles have been published on N-acetyltyrosine to date.

Especially glutaric acid, 2-hydroxyglutaric acid, benzoic acid, n-acetylaspartic acid, succinic semialdehyde-oxime, glycolic acid, 2-ketoglutaric acid, citric acid, methylmalonic acid, methylsuccinic acid, 3-hydroxyphenylacetic acid, 2-hydroxyisobutyric acid, homovanillic acid, 3,4-dihydroxyphenylacetic acid, and 2-ethyl-3-hydroxypropionic acid were decreased compared to normal people and reference values. Glutaric acid and 2-hydroxyglutaric acid are metabolites of lysine, hydroxylysine, and tryptophan amino acids, and a decrease was detected in the urine [18].

In addition, a peak was detected in all COVID-19 patients, but not in the device library. Further studies are needed on this.

As a result, tiglylglycine, 3-phenyllactic acid, 4-hydroxyphenylpyruvic acid, palmitic acid, and 5-hydroxyindolacetic acid are the most increased organic acids in COVID-19 patients. It indicates a disruption in lipid metabolism. Tiglylglycine is a urinary compound that has been specifically shown as a potential biomarker of respiratory chain disorders. It was also detected at a high level in this study.

Ethical Approval:

This study was approved by the Kanuni Sultan Suleyman Research and Training Hospital, Istanbul (KAEK/ 2021.10.253), and the procedures were according to the ethical standards of the responsible committee on human experimentation. Written informed consent was obtained from patients who participated in the study.

Declaration of Interest:

The authors declare that they have no potential conflicts of interest with respect to the research, authorship, and/ or publication of this article.

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