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ORIGINAL ARTICLE

Urine amino acid and gamma aminobutyric acid level in COVID 19 patients

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ABSTRACT

Background and Objectives: SARS CoV2 has tropism for various tissues, including the respiratory tract, brain, endothelium, heart, kidney and liver. Neurological symptoms can also be seen in the clinical course of the disease, and anosmia is the most common. The main objective of our study was to examine the urinary amino acid profiles of moderately severe patients diagnosed with COVID-19 with a positive RT-PCR test and try to find metabolic changes associated with the infection. Also, it was aimed to investigate the neuroinhibitory Gamma-Aminobutyric acid (GABA) levels in order to examine the physiopathology.

Materials and Methods: Thirty adult cases who were followed up in the infection clinic with positive SARS CoV 2 RT-PCR and diagnosed with COVID 19 disease were included in the study with consent. The amino acid profile of these patients' urine samples, 30 different amino acid levels and creatine levels were examined using the liquid chromatography-mass spectrometry (LCMS) method on the SCIEX QTRAP 4500 device.

Results: The mean age of the patients is 40 ± 5. Elevated GABA in 28/30 cases, high hydroxylysine amino acid in 27/30 cases, low glycine in 30/30 cases were detected in the urine. The creatinine levels of the patients were found to be normal.

Conclusion: It has been thought that the height of GABA may be due to bacteria producing GABA as a result of the change in microbiota due to lactic acidosis, as well as that the virus may directly affect the brain and cause an increase in GABA.

Keywords: COVID 19; Urin amino acids; Gamma-aminobutyric acid

INTRODUCTION

The Coronavirus disease 2019 (COVID-19) epidemic, which emerged in Wuhan, China in December 2019, spread rapidly around the world, causing a global pandemic (1). The COVID 19 pandemic affected the whole world, causing deaths, permanent sequelae, psychiatric and neurological com-

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plications. Despite primarily being a lung disease, it can produce devastating disease states that affect multiple organ systems, including the central nervous system (CNS). Other neurological conditions associated with COVID-19 include encephalopathy, anosmia, encephalitis, psychosis, brain fog, headache, depression, and anxiety (2). Although there are several hypotheses reported in the literature, a unifying physiopathological mechanism of most of these disorders remains unclear. Pulmonary dysfunction leading to insufficient oxygenation of the brain may explain encephalopathy and other disorders in COVID-19 patients. The angiotensin converting enzyme 2 (ACE2) receptor provides an entry route for the coronavirus to infect human host cells. These receptors are mainly located in the brain stem and are responsible for the regulation of cardiovascular and respiratory function. Like both Severe Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), COVID-19 can enter the brain directly via the olfactory pathway without the need for ACE2 receptors (3, 4). Direct invasion of the CNS by the virus or disruption of the blood-brain barrier by systemic cytokines released during infection may be responsible for these conditions (5). Especially anosmia is the most common neurological symptom (6, 7). SARS-CoV-2 infection causes a series of interactions between metabolic disorders (including demyelination) affecting neurotransmitters (acetylcholine, dopamine, serotonin and GABA), enkephalins and neurotrophins, chronic and acute inflammation, encephalopathy, cerebral ischemia and neurodegeneration. Most of the neurological manifestations of COVID-19 result from disturbances in choline-dependent anti-inflammatory signaling pathways, resulting in impaired β-endorphin activity and homeostasis of dopamine, serotonin, and GABA (8). GABA is widely distributed throughout the central nervous system (CNS) and is the major inhibitory neurotransmitter in the brain. Decreased GABA levels in the brain or impaired GABA function have been associated with psychiatric and neurological disorders such as anxiety, depression, insomnia and epilepsy. An increase in GABA leads to an inhibitory effect.

Metabolic analyses are a field of research that sheds light on pathophysiological mechanisms by profiling selected molecules and metabolites in the body. SARS-CoV-2 infection itself can cause major disturbances in the metabolite profile of infected individuals. The aim of this study was to analyze amino acid changes in the urine of patients during the acute phase of COVID-19. While previous studies examined changes in GABA observed in plasma in post-COVID syndrome, this study specifically aimed to examine its levels in acute disease state and in urine (9, 10). GABAergic, glutaminergic neurons and metabolic pathways are shown in Fig. 1.

MATERIALS AND METHODS

Clinical sample. Urines of 30 (thirty) hospitalized patients with positive COVID 19 RT PCR test in the infection clinic of our hospital were collected and evaluated in the laboratory by liquid chromatographic method (LS/MSMS). The study focused on patients with typical symptoms of SARS-CoV-2 infection (fever, cough, and dyspnea) with the aim of achieving a high level of homogeneity (in terms of means of COVID-19 presentation) within the cohort. Therefore, only hospitalized patients with moderate COVID-19 course, requiring oxygen supplementation but not invasive artificial pulmonary ventilation (according to National Institutes of Health/NIH/criteria) were included. Pediatric patients, intensive care patients and outpatients were excluded. People who were suspected of being COVID 19 positive, had recently had COVID 19, and people with any metabolic disease were excluded. Consent form was filled and signed by the patients. The urine profile of each patient was evaluated statistically by looking at 30 amino acids. It was a prospective observational study.

RT PCR protocol. The SARS CoV2 detection was performed by the COVID 19 qPCR test Direct Detect SARS CoV2 qPCR (RTA kitLtd) on BioRad CFX 96 platform (California, USA) according to the protocols provided by the manufacturer. The qPCR kit targets ORF1ab and the N gene of COVID 19 and the human RNaseP gene.

Urine amino acid procedure. Amino acid profile was evaluated by Liquid chromatography-mass spectrometry SCIEX QTRAP 4500 LCMS instrument. An internal standard containing deuterium standard was placed on the samples. (t-M histidine, alanine, arginine, aspartic acid, citrulline, cystine, glutamine, glutamic acid, homocystin, losin, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine have dotorium standards

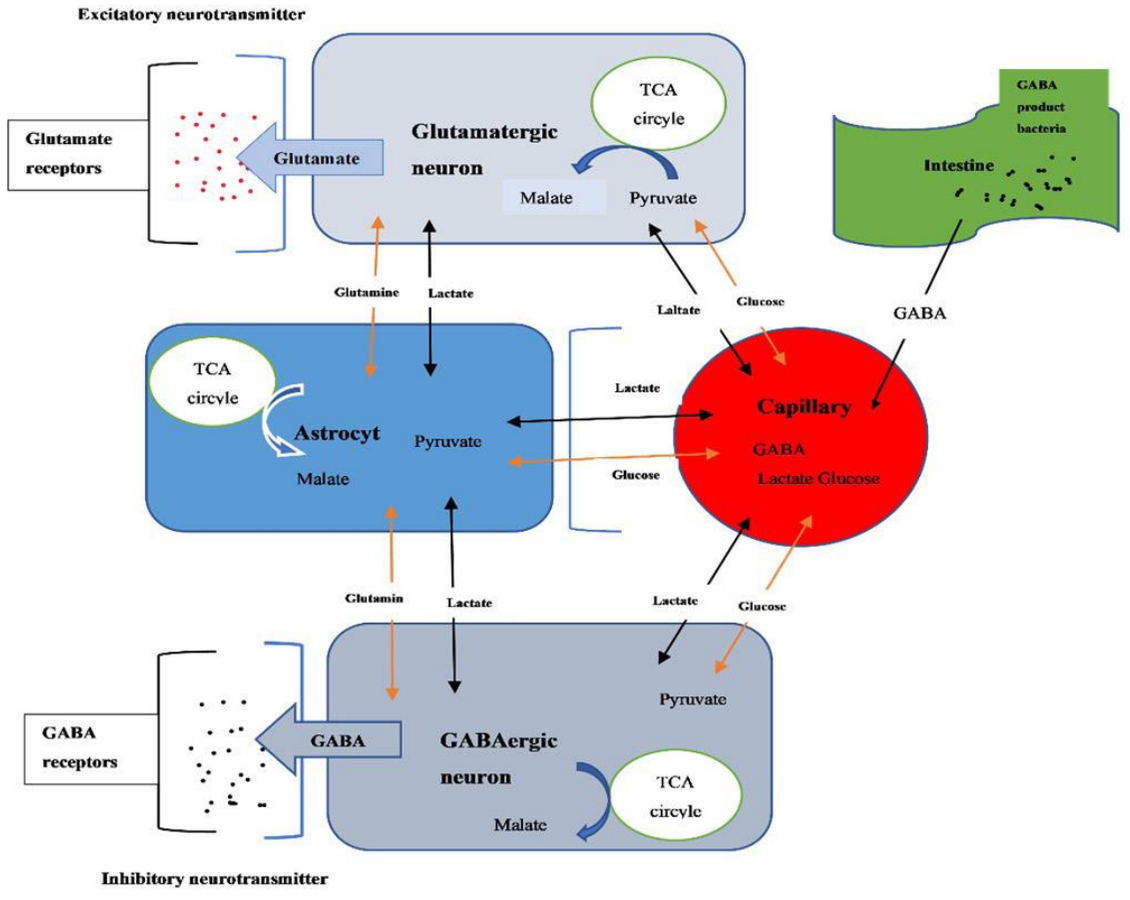

Fig. 1. Exogenous and endogenous sources of GABA are shown in Fig.

for 20 amino acids). Afterwards, acetonitrile proteins were precipitated. Samples were vortexed and incubated at 2-8°C. They were centrifuged at the end of the incubation. The supernatant was taken into the insert vial and given to the device for injection. (Liquid chromatography-mass spectrometry (LCMS) conditions: total Flow: 0.5 µl/min, mobile phase A concentration: 48%, mobile phase B Concentration: 54%) 30 amino acids were studied.

Statistical analysis. NCSS (Number Cruncher Statistical System) program was used for statistical analysis. Descriptive statistical methods (minimum, maximum, mean, median, standard deviation, frequency, percentage) 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 mean age and standard deviation of the 30 patients included in the study was 40 ± 5. Fifteen patients had anosmia. The urine creatinine levels of all the patients were found to be normal. Elevated GABA (27/30) and hydroxylysine (28/30), also low glycine (30/30) levels were found in the urine of patients. Urine amino acids mean, median, standard deviation, low, normal, and high patient numbers are given in the Table 1 and Fig. 2.

DISCUSSION

Examining the levels of metabolites in urine, which fluctuate due to interactions between microbial metabolites and the host, is crucial, particularly for diseases like COVID-19 that involve complex biological challenges. Understanding metabolic pathways is necessary to aid clinical research aimed at therapeutic development, comprehend disease mech-

Table 1. Distribution of urine amino acid

Amino acid	Mean ± SD	Referance value (µmol/gcrea)	Median (min-max)	Low n (%)	Normal n (%)	High n (%)
1-methyl-L-histidine	532 ± 194.48	0-1339	501.13 (256.29-1029.98)	0 (0.0)	30 (100.0)	0 (0.0)
3 -methyl-L-histidine	227.53 ± 254.58	0-500	149.91 (17.30-1156.15)	0 (0.0)	25 (83.3)	5 (16.7)
Alanine	470.87 ± 424.16	0-700	284.09 (72.56-1486.89)	0 (0.0)	24 (80.0)	6 (20)
Arginine	39.96 ± 36.27	0-114	26.81 (5.36-137.31)	0 (0.0)	27 (90.0)	3 (10)
Asparagine	176.42 ± 192.61	0-500	86.02 (19.35-772.26)	0 (0.0)	27 (90.0)	3 (10)
Aspartic acid	13.42 ± 12.61	0-100	8.48 (2.09-56.08)	0 (0.0)	30 (100.0)	0 (0.0)
Citrulline	61.03 ± 87.8	0-100	35.85 (3.42-488.69)	0 (0.0)	26 (86.7)	4 (13.3)
Cystine	99.99 ± 82.53	0-200	77.54 (15.05-358.89)	0 (0.0)	26 (86.7)	4 (13.3)
Cystathione	32.63 ± 54.67	0-30	18.48 (3.11-294.92)	0 (0.0)	22 (73.3)	8 (26.7)
GABA	21.99 ± 18.1	0-5	18.77 (2.6-101.58)	0 (0.0)	2 (6.7)	28 (93.3)
Glutamine	357.07 ± 287.75	93-686	251 (26.39-1004.61)	4 (13.3)	21 (70.0)	5 (16.7)
U-glutamic acid	27.83 ± 19.35	0-200	26.09 (8.73-110.16)	0 (0.0)	30 (100.0)	0 (0.0)
Glycine	234.02 ± 182.58	2229-2989	194.76 (32.37-762.08)	30 (100.0)	0 (0.0)	0 (0.0)
Homocystine	0.54 ± 0.65	0-10	0.37 (0.03-3.48)	0 (0.0)	30 (100.0)	0 (0.0)
Histidine	685.39 ± 574.86	0-1600	536.56 (19.53-2345.23)	0 (0.0)	27 (90.0)	3 (10.0)
Hydroxyproline	4.86 ± 6.28	0-15	2.54 (1.25-27.03)	0 (0.0)	27 (90.0)	3 (10.0)
Isoleucine	31.41 ± 22.08	0-100	25.49 (7.86-97.25)	0 (0.0)	30 (100.0)	0 (0.0)
Leucine	73.55 ± 586.85	0-100	46.54 (11.32-256.75)	0 (0.0)	22 (73.3)	8 (26.7)
Lysine	368.31 ± 586.85	0-271	198.1 (28.28-2948.69)	0 (0.0)	21 (70.0)	9 (30.0)
Methionine	20.26 ± 14.45	0-100	15.2 (1.71-56.77)	0 (0.0)	30 (100.0)	0 (0.0)
Hydroxylisine	181.27 ± 129.64	0-50	147.79 (11.63-578.32)	0 (0.0)	3 (10.0)	27 (90.0)
Ornithine	44.49 ± 58.08	0-150	26.36 (5.46-276)	0 (0.0)	28 (93.3)	2 (6.7)
Phenylalanine	94.41 ± 66.44	0-150	74.71 (12.65-300.73)	0 (0.0)	24 (80.0)	6 (20.0)
Proline	31.16 ± 77.12	0-100	12.49 (3.29-427.14)	0 (0.0)	29 (96.7)	1 (3.3)
Serine	332.57 ± 245.48	0-800	265.73 (48.83-1026.91)	0 (0.0)	28 (93.3)	2 (6.7)
Taurine	251.81 ± 205.88	0-1531	186.81 (12.89-713.93)	0 (0.0)	30 (100.0)	0 (0.0)
Threonine	433.1 ± 452.16	0-500	236.79 (35.41-1822.2)	0 (0.0)	22 (73.3)	8 (26.7)
Tryptophan	83.09 ± 53.86	0-150	69.45 (8.88-207.05)	0 (0.0)	26 (86.7)	4 (13.3)
Tyrosine	145.62 ± 116.82	0-200	115.43 (17.39-544.69)	0 (0.0)	23 (76.7)	7 (23.3)
Valine	83.9 ± 80.59	0-120	67 (13.38-423.97)	0 (0.0)	25 (83.3)	5 (16.7)

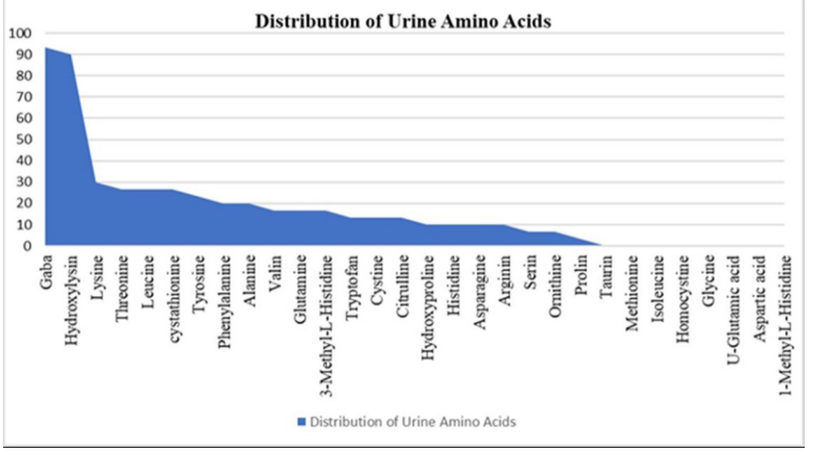

Fig. 2. Distribution of urine amino acid

anisms, and prevent related complications. Urinary amino acids are influenced by various factors, including diet, medication metabolism, interactions between the host and gut microbiota, normal physiological processes, and metabolic changes caused by COVID-19. This research explores the alterations in urinary amino acid levels in hospitalized COVID-19 patients, placing these changes within their broader metabolic framework.

In the literature, different results were obtained in studies where metabolic analyzes were performed in plasma and urine in COVID 19 (11, 12). Since metabolic analyses reflect the patient's current condition, it is important to evaluate each study on its own. In particular, we discuss the possible mechanisms of GABA, which we found at high levels in urine amino acid analysis. Secondly, we speculate on the possible causes and consequences of the high hydroxylysine and lysine levels and the low glycine levels.

The high level of GABA may occur as a result of the direct effect of the virus on the brain. Studies have shown that during brain activation in connection with transient increases in lactate, the uptake of Ca^{2+} into mitochondria and inhibition of the malate-aspartate shuttle between mitochondria and cytosol affect the glutamate/GABA-glutamine cycle (13). In a different study it was reported that chronic activation of immunological responses and increased glutamate/GABA ratio may lead to non-optimal organism functioning, leading to constant anxiety and keeping the body in an anxious state (14). In the study of Masoodi et al., plasma GABA level was found to be low in COVID 19 patients (15). In our study, GABA level in urine was also found to be high in 28/30 patients. GABA is an inhibitory neurotransmitter. In other studies, it was suggested that the neurological effects of the virus, even anosmia, may be related to the direct effect of the brain or the high GABA. The most typical neurological manifestations of COVID 19 are ischemia, neurodegeneration and/or anosmia/ageusia due to increases in proinflammatory cytokine levels (9, 16). It triggers COVID-19, ischemic stroke, Guillain-Barré syndrome, parkinsonism, polyneuropathy, meningitis, encephalitis, meningitis. Coronavirus infection also increased the severity of myopathies and multiple sclerosis (8, 17, 18). There are studies showing that there is an increase in GABA as a result of the change in the microbiota related to the lactic acidosis due to COVID 19. GABA-producing microorganisms (19) includ, *Bifidobacterium adolescentis* (DPC6044), *Bifidobacterium infantis* (UCC35624), *Lactobacillus brevis* (DPC6108), *Bifidobacterium dentium* (DPC6333) (20), *Lactobacillus plantarum* (ATCC14917) (21), *Lactobacillus rhamnosus* (22), and *Streptococcus salivarius* subsp. *thermophilus* Y2 (23). Is the predominance of bacteria producing gaba in COVID 19, due to lactic acidosis? Or is it that people with these bacteria have severe disease by increasing gaba production? Chicken or egg, egg or chicken? the question can be asked. Pyridoxine (vit 6), manganese, taurine and lysine increase the synthesis and effect of GABA. GABA is mainly metabolized by transamination to succinic semialdehyde. Succinate semialdehyde can either be reduced to γ-hydroxybutyrate by reaction catalyzed by L-lactate dehydrogenase or oxidized to succinate, an intermediate of the citric acid cycle, and from there to CO_2 and H_2O.

In our study, hydroxylysine and lysine levels were found to be higher than reference values. Hydroxylysin and lysine cross-link between tropocollagens and their high detection in urine can be explained by disruption in the collagen pathway. It may explain myopathies in COVID 19 patients. Collagenous colitis presented as a case is the evidence of degradation in collagen (24). Glycine has many different functions in the body; hemoglobin production, improving brain activity, strengthening immunity, restoration of the central nervous system, promoting the elimination of toxins, formation of connective tissue protein, lowering the pH in the body, normalization of blood glucose, glutathione production, and collagen production.

CONCLUSION

COVID 19, causes changes in immunological, hematological and metabolic pathways. The increase in GABA level is associated with neurological symptoms, especially anosmia. In addition, the elevation of hydroxylysin and lysine explains myopathies.

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