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Urea Cycle and Arginine Metabolic Changes in COVID-19 Patients

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Abstract

Aim: Metabolic changes begin after the invasion of an infectious microorganism and continue to develop as a series of interrelated events. Arginine is important in infectious diseases due to lymphocyte proliferation, nitricoxide production by macrophages, and the use of polyamides in the immune response. In this study, we aimed to examine the possible causes and consequences of urea cycle amino acid metabolism changes by comparing plasma arginine and urea cycle amino acid levels in Coronavirus disease-2019 (COVID-19) patients.

Materials and Methods: In this cross-sectional study, we evaluated the urea cycle and arginine metabolic changes and compared the plasma aminoacid levels of 35 COVID-19 patients and a healthy control group (n=35). The patient was diagnosed by reverse transcriptase-polymerase chain reaction of oropharyngeal-nasofaringeal swab specimens. For statistical analyzes, Mann-Whitney U and chi-square tests were used.

Results: The aminoacid plasma levels of argininosuccinate (1.03 µmol/L, $p=3.3x10^{-3}$), arginine (53.64 µmol/L, $p=1.1x10^{-3}$), aspartic acid (3.83 µmol/L, $p=5.5x10^{-9}$), citrulline (27.79 µmol/L, $p=3.3x10^{-5}$), glutamine (489.6 µmol/L, $p=9.0x10^{-17}$), lysine (206.4 µmol/L, $p=5.8x10^{-8}$), ornithine (129.5 µmol/L, p=0.012), plasma levels and glutamine/glutamate ($p=3.4x10^{-11}$), arg/ornithine (p=0.033), asp/argininosuccinate (p=0.011) ratios were decreased in the COVID-19 patient group compared to the healthy group.

Conclusion: Arginine is significant in endothelial control, the urea cycle, and immune activation. Arginine deficiency in COVID-19 patients may cause disturbances in this biological process and its pathways. As indicated by many clinical trials, we believe that preventing a decrease in plasma arginine levels will prevent a poor prognosis of patients and metabolic pathway disturbances in the urea cycle.

Keywords: Urea cycle, arginine, COVID-19, glutamine, citrulline, ornithine, virus disease

Introduction

In the last 3 years, the outbreak of the Coronavirus disease-2019 (COVID-19) infectious disease, which occurred at the end of 2019 just before the biggest Chinese festival, has significantly impacted the lives of millions of people. This disease has strained our medical and public health facilities worldwide. Also, the disease has caused compelling conditions for the world economy, people's living standards-psychological conditions that have prompted the need for urgent drug and vaccine treatment development (1).

Treatment with COVID-19 generally consists of case detection, isolation, monitoring, the prevention of infection, and supportive care treatments (2). Especially in infectious diseases, metabolic changes begin within hours after the invasion of an infectious microorganism and continue to develop as a series of interrelated events.

These responses vary in timing and magnitude with the clinical stages and severity of the disease (3).

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While some metabolic responses are seen in the initial phase of fever, subsequent responses can be seen at the onset of the convalescence stage. The response can be triggered by the direct effect of a microorganism or its toxic products on body cells. There are also secondary reasons for metabolic changes due to febrile, cardiovascular, inflammatory, or nutritional manifestations of an infectious process. At the end of the disease persistence, metabolic changes are variable (4).

Traditionally, amino acids have been classified into four different groups: essential, non-essential (the ability to be synthesized endogenously), based on their availability in an organism, their R group, or their metabolic products (ketogenic, glycogenic, and both glycogenic and ketogenic) (5). Plasma levels of arginine, a semi-essential amino acid, are met through diet, endogenous synthesis, or protein turnover (6). Arginine is used as a substrate in the metabolism of many biomolecules [agmatine, glutamate, glutamine, ornithine, creatinine, nitric oxide (NO) polyamines] and ammonia in our body (7). In healthy adults, dietary intake is not required as its endogenous synthesis is sufficient. However, during inflammation, infections, kidney and small intestine diseases, endogenous synthesis may not be sufficient to meet metabolic demands. Therefore, arginine homeostasis is modulated by arginine catabolism rather than arginine synthesis (8).

In the literature, it has been shown that plasma arginine levels decrease in infectious diseases due to lymphocyte proliferation, NO production by macrophages, and the use of polyamides in the immune response (9,10).

Studies have shown that the main protective factors in the immune system, NO synthesis (NOS), development and antibody production of B cells, and T cell receptor expression are modulated by arginine (11).

Also, it has been shown that the urea cycle amino acid metabolism in mammals is centered around L-arginine, and several other pathways involving arginine synthesis or catabolism enzymes are present within the urea cycle (12).

In this study, we aimed to examine the possible causes and consequences of urea cycle aminoacid metabolism changes by comparing the plasma arginine and urea cycle amino acid levels in COVID-19 patients (who were treated in the clinics with a similar diet) and healthy individuals according to Figure 1.

Materials and Methods

Patients Inclusion Criteria

Blood samples were taken from 35 patients who attended Sivas Cumhuriyet University or State Hospital Emergency Department with COVID-19 between 1 May-1 June 2020. The patient was

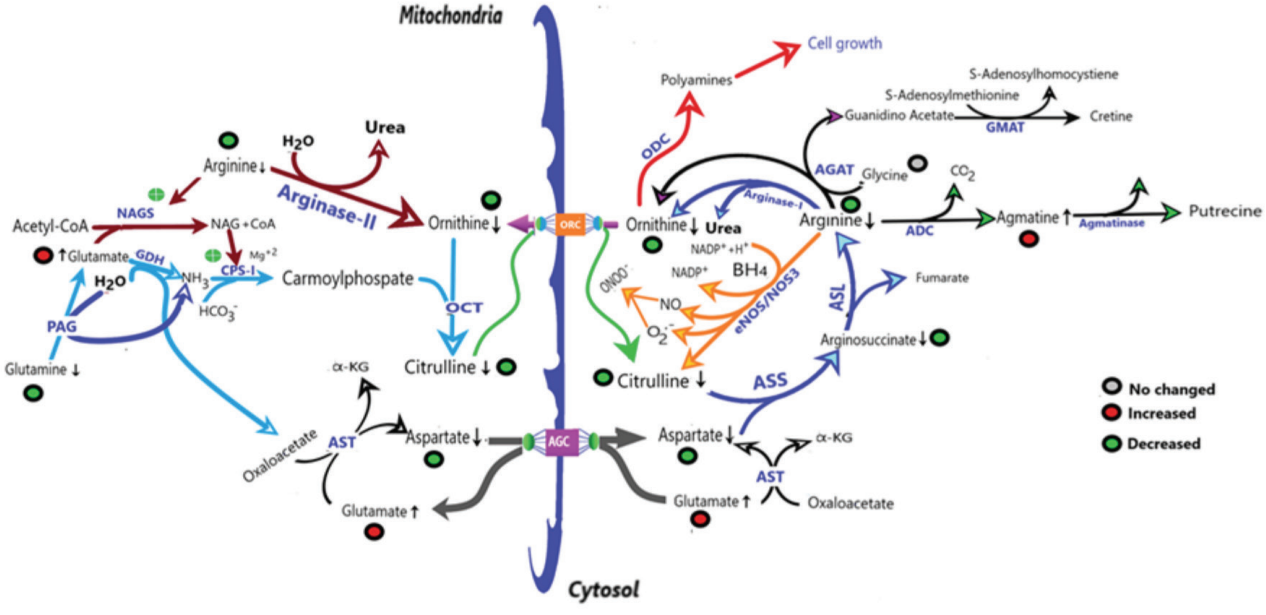

Figure 1. Urea cycle and aminoacid level alterations in COVID-19 patients. Ammonia metabolism is affected as urea cycle amino acids are significantly reduced in COVID-19 patients

COVID-19: Coronavirus disease-2019, NAGS: N-acetylglutamate synthase, AGAT: Arginine:glycine amidinotransferase, GAMT: Guanidinoacetate N-methyltransferase, eNOS: Endothelial nitric oxide synthase, ADC: Arginine decarboxylase, ODC: Ornithine decarboxylase, OCT: Ornithine transcarbamylase, GDH: Gluthamate dehydrogenase, PAG: Phosphate-activated glutaminase, ORC: Ornithine carrier, AGC: Aspartate-glutamate carrier, CPS 1 and CPS 2: Carbamoyl-phosphate synthetase 1 and 2

diagnosed by reverse transcriptase-polymerase chain reaction according to the oropharyngeal-nasofaringeal swab specimens. The results of the blood samples were compared with those obtained from 35 healthy volunteers who did not have any systemic disorders and were similar to the patient group in terms of gender and age.

Our study was derived from "determination of changes in plasma amino acid level in COVID-19 patients", which was approved by the Sivas Cumhuriyet University Interventional Ethics Committee with the decision number: 2020-04/02, date: 28.04.2020.

Exclusion Criteria

Patients who were alcohol and substance addicted, had chronic or acute disease (hypertension, chronic kidney failure, diabetes mellitus, liver damage, etc.), autoimmune disorders, or infectious disease were excluded from the study. Patients who were admitted to the intensive care unit due to COVID-19 disease were excluded because the dietary supplements given to intensive care patients will change the amino acid levels of the patient, which may cause false results in our study.

Samples

5-mL venous blood samples were taken from patients/healthy volunteers in lithium heparinized tubes and centrifuged for 5 min at 4000 rpm in a centrifuge. The resulting plasma was alquited into the Eppendorf tubes and stored at -80 °C until testing.

After we had achieved the required number of patients for the study, all the samples were defrosted and the amino acid levels were measured with a quantitative amino acid analysis kit using liquid chromatography mass spectrometry method.

Statistical Analysis

Data statistical analysis was performed using Statistical Package for the Social Sciences (version 23.0) licensed by our university. The Mann-Whitney U test is used to compare differences between two independent groups when the dependent variable is either ordinal or continuous but not normally distributed. The chi-square statistic is used for testing relationships between categorical variables. In our statistical analyzes an alpha of 0.05 was used as the cutoff for significance.

Results

The average ages of our patient and healthy control groups were 48.5±14.9, 48.8±14.6 years, respectively. While in the patient group, 65.7% (23) were male and 34.3% (12) female, in the healthy control group, 62.9% (22) were male and 36.1% (13) female. There was no statistically significant difference between the groups according to age (p=0.936) and gender (p=0.685).

The analyzed amino acid plasma levels of argininosuccinate, alanine, arginine, aspartic acid, citrulline, glutamine, lysine, orntine, proline plasma levels, and glutamine/glutamate, arginine/ornithine ratios were significantly decreased in the COVID-19 patient group compared with the healthy group. Glutamate, glycine, and agmatine levels were significantly higher in the COVID-19 patient group.

Discussion

In our study, we analyzed the aminoacid urea cycle changes among COVID-19 patients by comparing them with the control group. The urea cycle components (arginine, aspartate, citrulline, argininosuccinate, ornithine) and arg/ornithine and asp/argininosuccinate ratios decreased significantly in our study.

Metabolic disorders of amino acids follow viral infection diagnosis. These metabolic disorders may occur at the onset or during the infection period. Patients with excessive protein metabolism during the disease state have decreased immunity, increased infections and worsened outcomes (13,14).

L-arginine levels in the body have a main function in the normal immune system. In an immune response state, arginase can be released from certain granulocyte subsets and immature myeloid cells locally or systematically. This results in a decrease in plasma arginine level and an immune response to viral disease (15-17). Geiger et al. (18) determined that elevated L-arginine levels induced glycolysis and oxidative phosphorylation in activated T cells and endowed them with higher survival capacity through the generation of central memory-like cells. Zhu et al. (19) determined that physical injury decreased intracellular arginine in T cells, resulting in the inhibition of *in vivo* T-cell proliferation, memory, and cytotoxicity. This exponentially increased bacterial growth and mortality. Rees et al. (20) reported that the arginine and arginine to ornithine ratios have decreased significantly in both pediatric and adult Severe acute respiratory syndrome-Coronavirus-2 patients, which may contribute to T cell dysregulation, endothelial dysfunction, and coagulopathy.

In our study, the plasma arginine levels and arginine-to-ornithine ratio of COVID-19 patients decreased significantly compared with healthy volunteers, which is similar to the studies mentioned above.

Plasma arginine levels are determined by exogenous and endogenous sources. Arginine becomes a semi-essential amino acid during many stressful conditions, such as sepsis, and an arginine deficient state can be seen (21,22). Windmueller and Spaeth (23) were the first to demonstrate that the most important sources of endogenous arginine are synthesized from citrulline

(15% of the total arginine production) and protein catabolism (80% of the circulating arginine). Arginine is majorly obtained from citrulline via the small intestine-renal axis. The intestines are the most important source of plasma citrulline, not the liver. About 83% of the citrulline released by the intestines is taken up by the kidneys. About 75% of this citrulline is converted to arginine and released into circulation. The liver is a source of circulating citrulline only at high levels of ornithine and ammonia (24,25).

Glutamine accepted as a major precursor for citrulline synthesis in humans, supports citrulline production by increasing overall gut function. Windmueller and Spaeth (25) suggested that most of the circulating glutamine is converted to citrulline (about 28%) by the intestine. The decrease in plasma citrulline levels may be due to decreased glutamine levels, which may also be a cause of decreased arginine levels.

Our plasma arginine, citrulline, glutamine, and ornithine levels in COVID-19 patients decreased. However, there wasno statistical difference in the arginine/citrulline and glutamine/citrulline ratios between the patient and healthy volunteer groups (Table 1). Therefore, we thought that the intestine-renal axis was not affected. Also, the decreased level of our ornithine level reveals that the liver did not support the plasma citrulline level.

The urea cycle satisfies the body's demand for l-arginine. Citrulline consists of carbamoylphosphate and ornithine in the mitochondria by ornithine carbamoyltransferase. In the cytoplasm, it combines with aspartate and is converted to argininosuccinate by argininosuccinate synthetase. In the final step, arginine is synthesized by argininosuccinatelyase (Figure 1) (26). Nishio and Rehermann (27) suggested that interferons induced by viruses in viral diseases cause a decrease in the expression of urea cycle enzymes (ornithine transcarbamylase, carbamoyl phosphate synthetase 1, arginine succinate synthetase-1, and arginine succinate lysase) and increase the urea level by increasing the arginase-1 enzyme, suggesting that the hepatocytes are reprogrammed during the infection. They reported that this decreased plasma arginine level and the arginine/ornithine ratio and an increase in ornithine level. Caterino et al. (28) evaluated COVID-19 patients' serum metabolites and determined high ornithine levels in moderate and severe COVID-19 patients. whereas there wasno difference between the control and mild patient groups. Also, in their studies, sperm synthesis increased in COVID-19 patients. Masoodi et al. (29) evaluated lipid and amino acid metabolism in 19 COVID-19 patients. Plasma arginine, aspartate, citrulline, glutamate, glutamin, and ornithine levels decreased in COVID-19 patients compared with the control group.

Table 1. Plasma aminoacid levels in COVID-19 patients and control groups

Amino acid	Control groups				COVID-19 patients				
µmol/L	Mean	SD	Min-max		Mean	SD	Min	Max	p
Argininosuccinate	1.96	1.67	0.12	7.5	1.03	0.71	0.26	3.36	**$3.3x10^{-3}$**
Arginine	84.19	48.2	33.97	243.87	53.64	21.93	1.58	98.39	**$1.1x10^{-3}$**
Aspartic acid	14.06	8.86	3.44	42.25	3.83	1.96	1.03	6.96	**$5.5x10^{-9}$**
Citrulline	39.35	12.52	20.44	70.1	27.79	8.94	5.69	45.48	**$3.3x10^{-5}$**
Glutamate	102.1	30.89	53.3	162.42	176.5	57.75	68.48	301.1	**$4.4x10^{-9}$**
Glutamin	814.4	119.86	562	1062	489.6	126.73	240.2	735.2	**$9.0x10^{-17}$**
Glycine	316.1	82.66	159.1	510.93	341.2	95.41	169.5	587.5	0.243
Lysine	280.1	54.16	174.7	419.84	206.4	46.64	84.43	363.1	**$5.8x10^{-8}$**
Ornithine	151.3	38.92	97.26	260.52	129.5	31.17	64.82	183.4	**0.012**
Proline	358.7	108.36	153.2	613.78	298.3	113.38	130.6	636.8	**0.026**
Gln/glu	8.88	3.52	4.19	16.91	3.453	2.029	0.87	9.87	**$3.4x10^{-11}$**
Arg/lys	0.3	0.147	0.13	0.58	0.265	0.095	0.01	0.44	0.237
Arg/citrulline	2.39	1.52	0.66	6.91	2.31	1.64	0.05	7.92	0.848
Arg/ornithine	0.627	0.45	0.22	2.03	0.44	0.222	0.02	1.15	**0.033**
Asp/argininosuccinate	14.86	18.87	1.37	99.32	6.14	5.57	0.6	22.12	**0.011**
Citrulline/argininosuccinate	48.94	75.7	7.05	428.89	46.01	39.86	5.52	174.35	0.84
Glutamine/citrulline	22.43	6.56	10.56	39.7	20.949	14.68	7.19	89.79	0.587
Ornithine/citrulline	4.13	1.37	1.72	7.67	5.23	2.35	1.96	14.36	**0.02**
Agmatin (ng/mL)	40.1	8.37	26.79	51.42	46.55	15.99	24.68	67.72	**0.038**

COVID-19: Coronavirus disease-2019, SD: Standard deviation, Min-max: Minimum-maximum

In our study, the urea cycle components (arginine, aspartate, citrulline, argininosuccinate, ornithine) and arg/ornithine and asp/argininosuccinate ratios decreased significantly. There was a nonsignificant decrease in arg/citrulline and citrulline/argininosuccinate. whereas ornithine/citrulline (p=0.02) and argininosuccinate/arg (p=0.39) ratios increased. All of these deteriorations revealed that the urea cycle was affected in COVID-19 disease (Figure 1, Table 1). Our results are similar to those reported in the literature, except for the ornithine level, which has been increased in Nishio and Rehermann (27) and Caterino et al.'s (28) moderate and severe group studies. The difference for ornithine compared with Caterino et al. (28) level may be that all of our patients were clinically in the mild group and none of them were admitted to the intensive care unit. We determined an increase in agmatine levels in our patient group. The decrease in arginine because of agmatine conversion may be another reason for the low ornithine. Also, the increase in the spermin level from ornithine can decrease the ornithine level.

While the citrulline/argininosuccinate decrease was nonsignificant, the aspartate to argininosuccinate ratio decreased significantly. This suggests that the argininosuccinate synthase pathway was not affected, and partate may be used in nucleotide and different metabolic synthesis pathways. We determined that the decrease in arginine and the arginine/ornithine ratio are due to the increase in NOand agmatine synthesis. Because in our study, plasma agmatine levels were increased in the patient group compared with the healthy volunteers (Figure 1, Table 1).

Arginine plays an important role in the make-up of essential proteins, and various isoforms of NOS convert arginine into NO and citrulline (12). NOS is encoded by three isoenzymes in humans: neuronal NOS, inducible NOS (iNOS), and endothelial NOS. Arginine is metabolized by these three NOS enzymes and produces NO, which crucially participates in vasodilatation processes and cytotoxic mechanisms (30). Synthesized iNOS has endogenous antiviral effects by inhibiting viral enzymes through nitrosylation of viral proteins, damaging viral DNA via oxidative and nitrosative stress, modulating viral-encoded transcription factors, and further activating host signaling pathways for adaptive response (31).

The presence of sufficient arginine in the body is of vital importance in the immune response mediated by NO against viral infections. In our patient group, plasma arginin levels decreased, which suggests that arginin may be used for iNOS. This can disturb the urea cycle and may also be the reason for our urea cycle amino acid decrease.

Study Limitations

In our study, we could not examine the changes in the urea cycle and amino acid metabolism in clinically severe patients because only the patients who were clinically in mild condition and were followed up in the ward were evaluated. Conducting these studies will better reveal the importance of arginine deficiency and changes in the urea cycle for mortality prediction and prevention.

Conclusion

Arginine is significant in endothelial control, the urea cycle, and immune activation. Arginine deficiency in COVID-19 patients may cause disturbances in this biological process and its pathways. As many clinical trials have indicated, we think that preventing a decrease in plasma arginine levels will prevent the poor prognosis of patients and the problems they may encounter in metabolic pathways such as the urea cycle. Arginine is present in COVID-19, and preliminary results from a randomized clinical trial seem to support this view.

Ethics

Ethics Committee Approval: The study was approved by the Sivas Cumhuriyet University Interventional Ethics Committee (decision number: 2020-04/02, date: 28.04.2020).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.A., İ.K., Y.K.T., G.T., S.Y., A.B.S., Concept: H.A., İ.K., Y.K.T., G.T., Design: H.A., İ.K., Y.K.T., G.T., S.Y., Data Collection or Processing: H.A., İ.K., Y.K.T., Analysis or Interpretation: İ.K., Y.K.T., Literature Search: S.Ö., İ.K., Y.K.T., S.Y., A.B.S., N.H., Writing: H.A., İ.K., Y.K.T., G.T.

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