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*Original Article*

# New Biomarkers in the Diagnosis of COVID-19: Amino Acids

*COVİD-19 Tanısında Yeni Biyobelirteçler: Amino Asitler*

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## Abstract

**Background:** Coronavirus disease 2019 (COVID-19) is still spreading rapidly around the world, and early detection before its severe symptoms begin is important, but challenges in early detection remain. There is also very little data on the effects of COVID-19 on amino acid metabolism. The aim of this study is to analyze the free amino acid profile in the blood serum of COVID-19 patients and to determine their biomarker potential for diagnosis.

**Materials and Methods:** In this study, blood samples were taken from 30 COVID-19 patients and 30 healthy individuals, and their amino acid profile was analyzed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Biomarker and pathway analyzes from multivariate statistical analyzes were performed with the MetaboAnalyst program.

**Results:** Among the amino acids we analyzed, 6 of them were amino acids with strong potential to be used in the diagnosis of COVID-19. According to the AUC value (>0.800), these amino acids are alpha-aminoadipic acid, arginine, proline, alanine, sarcosine and citrulline, respectively. In addition, according to the pathway analysis, it was found that arginine and proline metabolisms were significantly impaired in COVID-19 disease ($p$<0.001 and impact value: 0.356).

**Conclusions:** This study showed that changes in the amino acid profile of COVID-19 patients may be important in diagnosis, treatment, and prognosis. According to our results, alpha-aminoadipic acid may be an excellent candidate for diagnosis.

**Keywords:** Amino Acid Profiling, Biomarker, COVID-19, MetaboAnalyst, Metabolomics

## ÖZ

**Amaç:** Koronavirus hastalığı 2019 (COVİD-19) hala dünya çapında hızla yayılıyor ve şiddetli semptomları başlamadan önce erken teşhis önemlidir ancak erken teşhisteki zorluklar devam etmektedir. Ayrıca COVİD-19'un amino asit metabolizması üzerindeki etkileri hakkında çok az veri bulunmaktadır. Bu çalışmanın amacı, COVİD-19 hastalarının kan serumundaki serbest amino asit profilini analiz etmek ve tanı için biyobelirteç potansiyellerini belirlemektir.

**Gereç ve Yöntem:** Bu çalışmada 30 COVİD-19 hastası ve 30 sağlıklı bireyden kan örnekleri alındı ve amino asit profilleri sıvı kromatografi-kütle spektrometresi/kütle spektrometresi (LC-MS/MS) ile incelendi. MetaboAnalyst programı ile çok değişkenli istatistiksel analizlerden biyobelirteç ve yol analizleri yapıldı.

**Bulgular:** Analiz ettiğimiz amino asitlerden 6 tanesi COVİD-19 tanısında kullanılabilecek güçlü potansiyele sahipti. AUC değerine göre (>0.800), bu amino asitler sırasıyla alfa-aminoadipik asit, arginin, prolin, alanin, sarkosin ve sitrülindir. Ayrıca yolak analizine göre COVİD-19 hastalığında arginin ve prolin metabolizmalarının anlamlı düzeyde bozulduğu bulundu ($p$<0,001 ve etki değeri: 0.356).**Sonuç:** Bu çalışma, COVİD-19 hastalarının amino asit profilinde meydana gelen değişikliklerin teşhis, tedavi ve prognozda önemli olabileceklerini gösterdi. Bizim sonuçlarımıza göre alfa-aminoadipik asit, tanı için mükemmel bir aday olabilir.

**Anahtar kelimeler:** Amino Asit Profili, Biyobelirteç, COVİD-19, MetaboAnalyst,

## Highlights

- Amino acid metabolism changes significantly in COVID-19 disease
- Amino acids may be new biomarkers in the diagnosis of COVID-19.

## Introduction

COVID-19, caused by infection with the Human Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), is expected to spread rapidly around the world and new global waves are expected (1). According to the data reported to the World Health Organization, 483.556.595 cases and 6.132.461 deaths have been reported worldwide. In addition, 11.054.362.790 doses of vaccine were administered (2). In many studies, it has been reported that people with obesity, diabetes, old age, male gender, cardiovascular disease and chronic disease show hypersensitivity to COVID-19, and their risk of death increases if they are infected with SARS-CoV-2. In addition, it has been observed that the symptoms (asymptomatic, mild, moderate or severe symptoms) of this disease show great variability (3, 4). The number of cases and deaths continues to increase worldwide as a result of the development of new variants or the decrease in the effect of drugs and vaccines on the virus. There is still a need for prognostic tests that can illuminate the effect of COVID-19 on host metabolism, stage the disease or predict its severity, and be used in early diagnosis (5, 6).

Reverse Transcription Polymerase Chain Reaction (RT-PCR) is still the main diagnostic method for COVID-19. However, under the current pandemic conditions, the sensitivity of RT-PCR is not high to keep the pandemic under control. Therefore, missing a case of COVID-19 in RT-PCR tests is a public health risk and will cause the pandemic to spread to wider areas (7). Computed Tomography (CT), another method used in the detection of COVID-19 pneumonia, causes the escape of infected people due to its failure in early diagnosis, cost, insufficient radiologists and time (7, 8). These shortcomings of diagnostic methods necessitated the discovery of new biomarkers. If the disease is diagnosed early, treatment can be planned early and deaths can be reduced. In this context, the metabolomics approach is recognized as a promising technique in the discovery of new biomarkers (1, 6, 9).

The metabolomics approach, which belongs to the science of omics, studies numerous endogenous or exogenous metabolites such as carbohydrates, lipids, nucleic acids, organic acids and amino acids (10). Various invasive and non-invasive biological samples such as blood, urine, saliva and cerebrospinal fluid are used in metabolomic analysis. However, blood better reflects the metabolomic profile in disease states (11). Identification of the metabolic pathway in the cell provides an understanding of the physiology of the cell and the general state of the organism (12). In addition, among the goals of metabolomics is to develop biomarkers that can be used in the evaluation of diagnosis, prognosis and therapeutic response (13). Amino acids are metabolites with high potential that can be used both in the explanation of the pathophysiology and in the diagnosis of diseases, since they are the building blocks of proteins, relationship with organ systems, and metabolic regulators of physiological states (14, 15).

The current study aimed to improve our understanding of the pathogenesis of the disease by analyzing the serum amino acid profile of COVID-19 patients with LC-MS/MS, and also to discover new biomarkers for early diagnosis of the disease before severe symptoms of the disease appear.

## Material and Method

### Study Design

This study, which complies with The Code of Ethics of the World Medical Association (Declaration of Helsinki), was approved by the local ethics of Harran University Faculty of Medicine (01 June 2020, session 10). All individuals participating in this study were given detailed information and written informed consent was obtained from all patients.

30 adult patients with positive SARS-CoV-2 RT-PCR test hospitalized in Harran University Medical Faculty Training and Research Hospital were included in the COVID-19 group. The control group was selected from healthy volunteers. Exclusion criteria were COVID-19 disease, acute and chronic diseases, contact with COVID-19 positive patients, and drug use.

### Sample Collection and Analysis of Amino Acid Profiles

Blood samples placed in anticoagulant tubes were centrifuged at 4000 rpm for 10 minutes at $4^0$C and, the supernatants (plasma) were transferred to new tubes and frozen in liquid nitrogen and stored at $-80^0$C until analyzed. The plasma amino acid amount was determined by LC-MS/MS according to the Jasem brand kit protocol. 50 µl of supernatant was added to a new tube, 50 µl of internal standard mix and 700 µl of Reagent-1 were added and vortexed for 10 seconds. It was centrifuged at 4000 rpm for 5 min. The obtained supernatant was transferred to HPLC vial and 32 amino acid species were analyzed in LC-MS/MS (Shimadzu 8045, Japan) device.

### Statistical Analysis

Data were statistically analyzed using IBM SPSS 25.0 and presented as mean ± standard deviation. Normality was evaluated with the Shapiro-Wilk test. The differences between the two groups were analyzed using Student's T-test and Mann-Withney U Test. A *p* value less than 0.05 was considered statistically significant.

Metabolite data obtained as a result of LC-MS/MS analysis were uploaded to MetaboAnalyst 5.0 server. First, partial least squares discriminant analysis (PLS-DA) was performed to determine the trends and clustering of individuals in the COVID-19 and control groups. Amino acids contributing to differentiation and clustering between groups were scored with variable significance in projection (VIP) analysis. A heatmap was created through hierarchical clustering, which allows visualization of the concentrations in groups of the 32 amino acids we analyzed. MetaboAnalyst's 'Biomarker Analysis' module based on the receiver operating characteristic (ROC) curve was used to identify potential biomarkers associated with COVID-19. In addition, pathway analysis was performed using MetaboAnalyst 5.0 and the Homo Sapiens-Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

## Results

In our study, the COVID-19 group consisted of 11 males and 19 females, and the control group consisted of 16 males and 14 females volunteers. The mean age in the covid-19 group was found to be statistically significantly higher than the control group ($p$<0.05) **(Table 1).**

**Tablo 1.** Demographics of participant.

| **Demographic Data** | **COVID-19** | **CONTROL** | ***P*** |
|---|---|---|---|
| Gender (Male/Female) | 11/19 | 16/14 | |
| Age (Years) | 34.6 ± 5.95 | 30.05 ± 4.43 | 0.01 |

32 amino acids were analyzed in the serum of patients and healthy controls. The mean concentrations and standard deviations of amino acids are shown in **Table 2**. According to the analysis results, the decrease in alanine, arginine, beta-aminoisobutyric acid, citrulline, cystine, ethanolamine, glycine, histidine, proline and sarcosine amino acids in the COVID-19 group compared to the control group was found to be statistically significant ($p$<0.05). In addition, the increase in alloisoleucine, alpha-aminoadipic acid, alpha-aminobutyric acid, isoleucine and ornithine amino acids was statistically significant in the COVID-19 group compared to the control group ($p$<0.05). However, the changes in the remaining 17 amino acids (asparagine, glutamic acid, lysine, methionine, serine, threonine, tryptophan, valine, aspartic Acid, beta-alanine, 1-Methylhistidine, gamma aminobutyric acid, glutamine, hydroxyproline, leucine, tyrosine, phenylanalanine) were not statistically significant ($p$>0.05).

## COVID-19 Patients Differentiated by PLS-DA

PLS-DA was performed to visualize the distribution of COVID-19 patients and healthy individuals in groups, and results were shown as two-dimensional (2D) and three-dimensional (3D) **(Figure 1).** According to the results of the analysis, it was seen that there was evident separation and clustering. The analyzed amino acids were considered successful in separating the two groups.

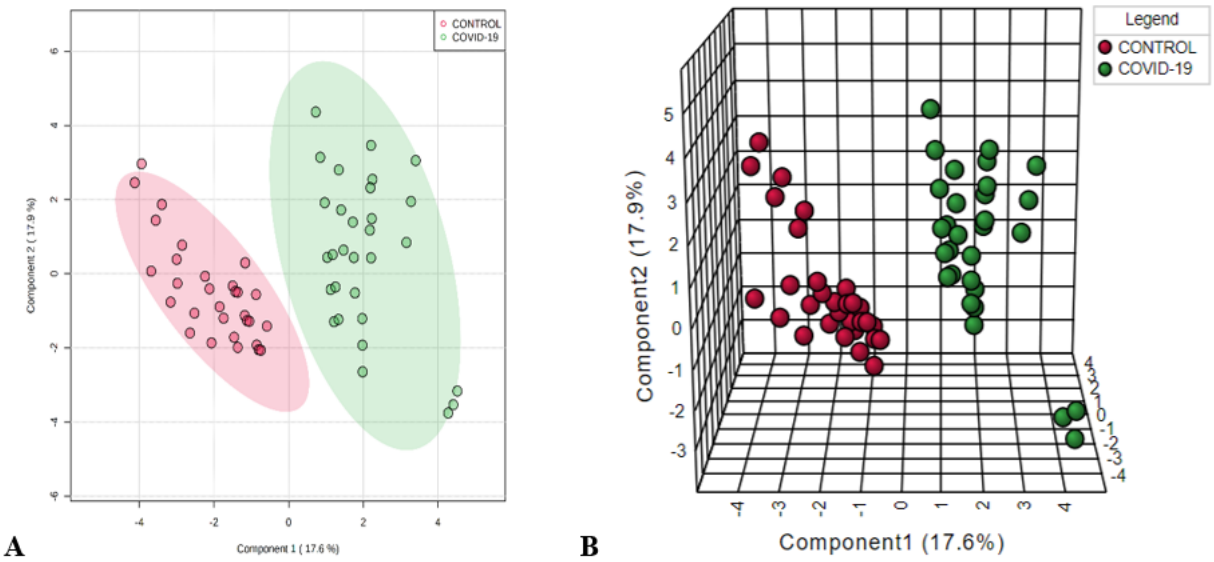


**Figure 1.** 2D (A) and 3D (B) score plots of PLS-DA analysis. Screening of Differential Amino Acids

**Table 2.** Quantitative analysis of serum amino acids composition of the groups.

| Amino Acid (µmol/L) | Abbreviation | COVID-19 | CONTROL | *p* |
|---|---|---|---|---|
| Alanine[a] | Ala | 339.65±89.47 | 478.90±110.49 | <0.001 |
| Alloisoleucine[a] | Allo-Ile | 0.68±0.13 | 0.62±0.08 | 0.027 |
| Arginine[a] | Arg | 66.80±19.85 | 101.88±15.66 | <0.001 |
| Asparagine[a] | Asn | 44.78±11.94 | 46.60±8.49 | 0.498 |
| Citrulline[a] | Cit | 24.47±10.44 | 39.45±14.54 | <0.001 |
| Glutamic Acid[a] | Glu | 143.28±68.20 | 127.56±40.01 | 0.281 |
| Histidine[a] | His | 76.43±17.92 | 93.45±17.47 | <0.001 |
| Isoleucine[a] | Ile | 80.49±16.17 | 72.61±10.69 | 0.030 |
| Lysine[a] | Lys | 185.23±33.67 | 169.45±38.28 | 0.095 |
| Methionine[a] | Met | 27.04±6.25 | 24.70±4.59 | 0.104 |
| Proline[a] | Pro | 172.84±53.57 | 276.44±75.23 | <0.001 |
| Sarcosine[a] | Sar | 27.15±7.77 | 36.83±7.28 | <0.001 |
| Serine[a] | Ser | 171.61±44.65 | 168.96±29.66 | 0.787 |
| Threonine[a] | Thr | 130.52±32.76 | 128.87±31.13 | 0.841 |
| Tryptophan[a] | Trp | 59.63±9.02 | 53.68±14.29 | 0.059 |
| Valine[a] | Val | 228.92±41.61 | 223.64±37.32 | 0.607 |
| 1-Metilhistidine[b] | 1-Mhs | 1.75±0.60 | 2.26±1.14 | 0.188 |
| Alpha-Aminoadipic Acid[b] | A-Aaa | 1.38±0.68 | 0.26±0.08 | <0.001 |
| Alpha-Aminobutyric Acid[b] | A-Aba | 20.13±7.69 | 12.89±3.80 | <0.001 |
| Aspartic Acid[b] | Asp | 41.46±11.98 | 45.85±15.01 | 0.478 |
| Beta-Alanine[b] | B-Ala | 3.25±1.30 | 3.36±1.38 | 0.201 |
| Beta-Aminoisobutyric Acid[b] | B-Aiba | 0.14±0.13 | 0.83±0.65 | 0.001 |
| Cystine[b] | Cys | 12.12±6.60 | 19.86±7.00 | <0.001 |
| Ethanolamine[b] | Eta | 13.93±8.97 | 19.44±8.67 | 0.022 |
| Gamma-Aminobutyric Acid[b] | GABA | 6.70±5.42 | 4.85±4.88 | 0.243 |
| Glutamine[b] | Gln | 600.26±180.33 | 586.94±164.08 | 0.701 |
| Glycine[b] | Gly | 341.82±80.36 | 410.33±118.05 | 0.031 |
| Hydroxyproline[b] | Hyp | 59.55±20.51 | 56.62±10.64 | 0.255 |
| Leucine[b] | Leu | 159.09±42.50 | 149.22±18.65 | 0.124 |
| Ornithine[b] | Orn | 79.26±31.34 | 60.67±23.24 | 0.008 |
| Phenylanalanine[b] | Phe | 106.20±27.96 | 111.36±16.91 | 0.101 |
| Tyrosine[b] | Tyr | 68.86±15.57 | 64.24±14.45 | 0.280 |

[a]Student's T-Test, [b]Mann-Withney U Test

VIP analysis ranks the amino acids that contributed to the differentiation of the control group and the COVID-19 group according to their contribution power (Figure 2). Alpha-aminoadipic acid, arginine and proline are the three amino acids with the highest VIP scores (>1.7). The higher the VIP score, the higher the contribution to separation. In addition, a heat map was drawn to visualize the densities of amino acids in both the COVID-19 and Control groups (Figure 3). In the heatmap, columns represent individuals in groups and rows represent amino acids. The brown and blue bands indicate the up- and down-regulation of amino acids, respectively. In addition, the increase and decrease of the depth of these colors express the increase and decrease of the regulation. Looking at the heat map, it is seen that the density of 17 amino acids increased and the density of 15 amino acids decreased relatively in the COVID-19 group.

**Biomarker Candidates for COVID-19**

ROC analysis was performed to identify potential biomarkers for COVID-19. As a result of the analysis, the AUC, cut-off value, sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), and p values of the 6 biomarkers with the highest AUC values are shown in Table 3. Our analyzes showed that alpha-aminoadipic acid, arginine and proline could be an biomarker for COVID-19 in the light of this information and the ROC curve is shown in Figure 4. Alpha-aminoadipic acid value higher than 0.694 µmol/L, arginine value lower than 81.6 µmol/L and proline value lower than 225 µmol/L showed that the person may have covid 19 disease.

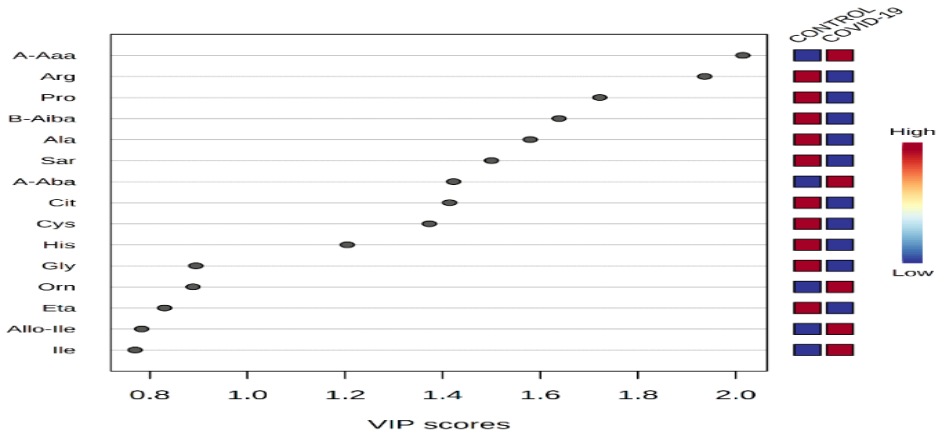


**Figure 2.** VIP score graph of serum amino acids.

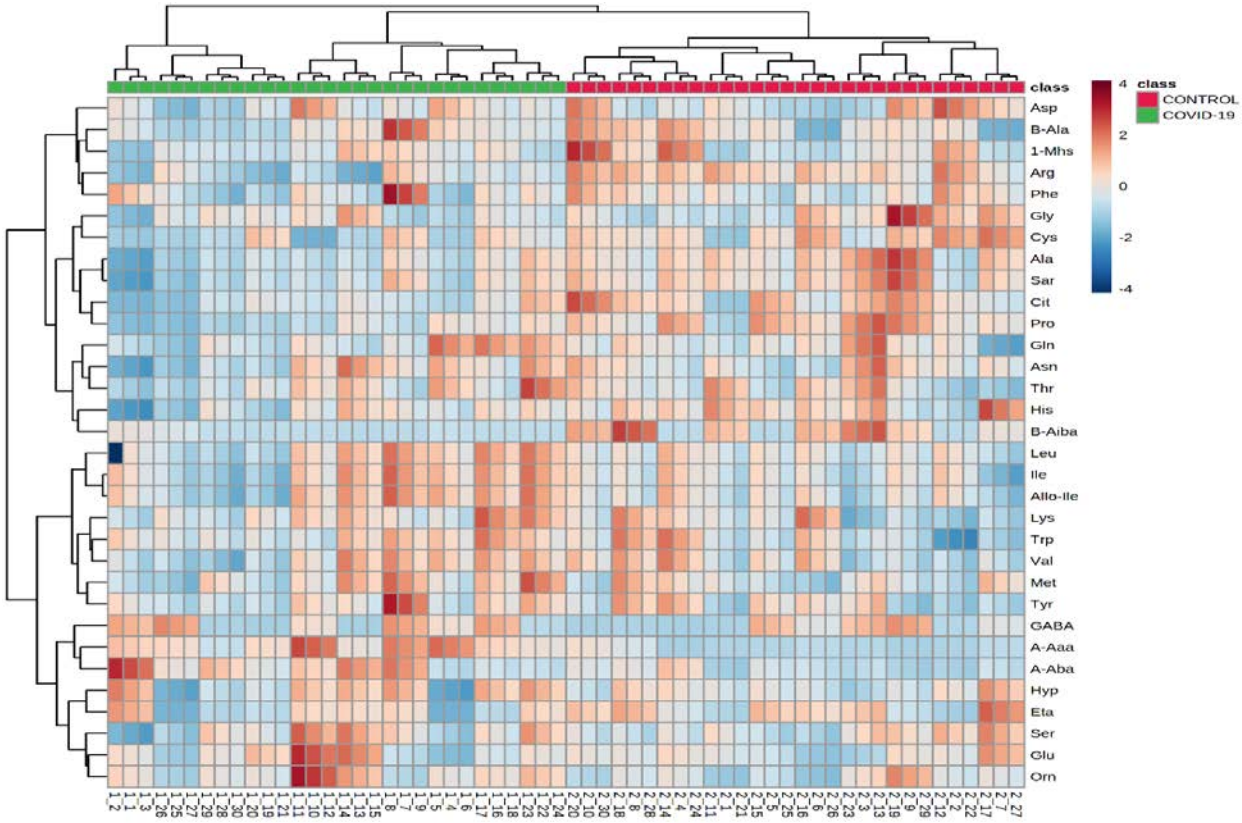


**Figure 3.** Heatmap: Amino acid concentration differences in groups.

**Table 3.** ROC analysis results

| Amino Acids | Cut-off | AUC | Sensitivity | Specificity | LR (+) | LR (-) | *p* |
|---|---|---|---|---|---|---|---|
| Alpha-Aminoadipic Acid | 0.694 | 0.964 | 0.90 | 0.9 | 9.00 | 0.111 | <0.001 |
| Arginine | 81.6 | 0.923 | 0.80 | 0.93 | 12.0 | 0.214 | <0.001 |
| Proline | 225 | 0.867 | 0.80 | 0.76 | 3.42 | 0.260 | <0.001 |
| Alanine | 417 | 0.841 | 0.80 | 0.76 | 3.48 | 0.260 | <0.001 |
| Sarcosine | 31.3 | 0.817 | 0.73 | 0.83 | 4.40 | 0.320 | <0.001 |
| Citrulline | 33.7 | 0.802 | 0.90 | 0.66 | 2.70 | 0.150 | <0.001 |

**Table 4.** Impaired metabolic pathway in the COVID-19.

| Pathway Name | Match Status | *P* | -log(*p*) | Impact |
|---|---|---|---|---|
| Arginine and proline metabolism | 5/38 | <0.001 | 17.352 | 0.356 |
| Aminoacyl-tRNA biosynthesis | 20/48 | <0.001 | 4.2154 | 0.166 |
| Beta-Alanine metabolism | 3/21 | <0.001 | 3.4011 | 0.399 |
| Alanine, aspartate and glutamate metabolism | 6/28 | 0.002 | 2.6597 | 0.621 |
| Glutathione metabolism | 4/28 | 0.009 | 2.0148 | 0.111 |
| Glycine, serine and threonine metabolism | 5/33 | 0.012 | 1.8998 | 0.555 |

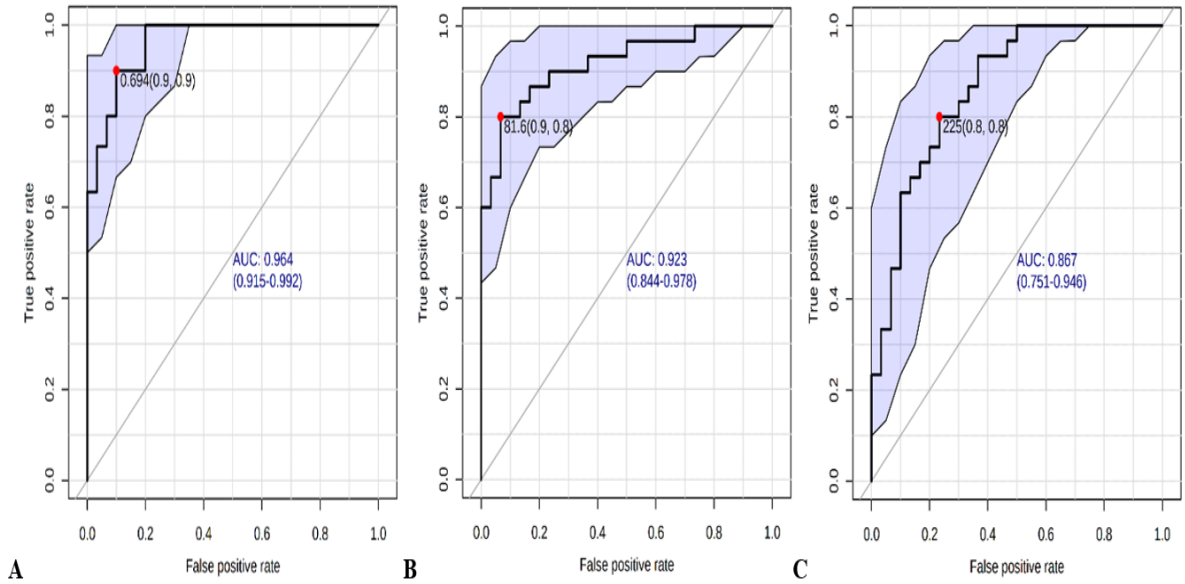


**Figure 4.** ROC curves of Alpha-aminoadipic acid (A), Arginine (B) and Proline (C). (Red dots show cut-off, sensitivity and specificity values)

## Pathway Analysis

The discovery of biological pathways may contribute to elucidating the altered metabolomic metabolism of humans infected with SARS-CoV-2. Analysis results showed the presence of significantly altered metabolites and biological pathways in COVID-19 patients. Disruption in 34 metabolic pathways has been identified. However, $p < 0.05$ or pathway impact > 0.1 indicates significantly altered pathway. Match Status, *p*-value, -log(*p*) and impact values of altered metabolic pathway ($p < 0.05$) are given in **Table 4.**

Pathway analysis (Impact>0.1 and $p<0.05$) showed 6 different pathways that changed significantly in COVID-19 (**Table 4 and Figure 5).** These; arginine and proline metabolism, aminoacyl-tRNA biosynthesis, beta-alanine metabolism, alanine, aspartate and glutamate metabolism, glutathione metabolism and glycine, serine and threonine metabolism. In addition, metabolic pathway with impact value > 0.1 and - log (*p*) > 10 are the pathway with the highest impared. According to this information, our analysis result showed that Arginine and proline metabolism was the most disrupted metabolic pathway.

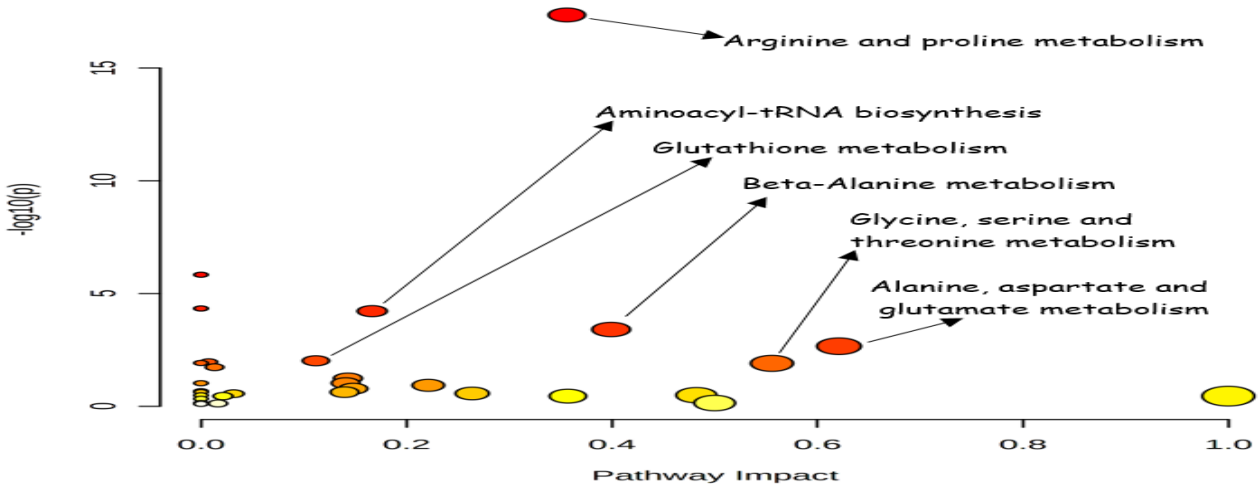


**Figure 5.** Summary of pathway analysis.

## Discussion

Metabolomics is a promising "omics" field in systems biology; Its purpose is the comprehensive analysis of low molecular weight endogenous and exogenous metabolites. Amino acids, organic acids, fatty acids and carbohydrates are important metabolites in the biological system. In addition, they are attractive candidates for understanding disease phenotypes, as they are end products of transcription and translation (16). Metabolomics approach could enable mapping of disruptions of early biochemical changes in diseases therefore, it may provide an opportunity to develop predictive biomarkers that could result in earlier intervention and provide valuable insights into the mechanisms of diseases (17). Despite difficulties in the selection of the mass spectrometry method and the evaluation of metabolomic data, it offers excellent potential in the discovery of diagnostic biomarkers for COVID-19 (18).

It is known that changes in amino acid concentrations in biological fluids provide information about many types of diseases such as diabetes mellitus, oncologic diseases (such as colorectal cancer, breast cancer, lung cancer, esophageal cancer, pancreatic cancer and prostate cancer), Alzheimer's disease, cardiovascular disease, bowel disease and obesity (19). In addition, the importance of the use of foods with high amino acid/protein content in the treatment was reported in order to strengthen immunity against COVID-19 and reduce the emerging symptoms (20). As a result of the data obtained, it has been suggested that amino acids can be used as a biomarker and treatment target in terms of diagnosing diseases and examining the effectiveness of treatment (21). In our study, three amino acids (Alpha-aminoadipic acid, Arginine ve Proline) were identified that change significantly in the serum of the participants, contribute the most to the separation of sick and healthy individuals (VIP scores > 1.7), and also have high potential (AUC>850) as diagnostic markers that can be used in the diagnosis of the disease.

First, Alpha-aminoadipic acid, also known as 2-aminoadipic acid, is a derivative of the amino acid lysine. Lysine, which is found in the structures of proteins, forms intermediate allysin by oxidation and deamination. However, if it undergoes further oxidation, alpha-aminoadipic acid is formed and passes from the tissues to the plasma as a result of the breakdown of proteins, as in other amino acids (22). Alpha-aminoadipic acid has been reported to be a biomarker with high potential for diseases with high morbidity and mortality in humans such as skin aging, renal failure, crystalline sclerosis, aging, and sepsis (23, 24). In a study by Borchers et al. in which biomarkers that can be used to determine the severity of COVID-19 disease were investigated, alpha-aminoadipic acid was reported to be a good biomarker. In our study, it was found to be significantly higher in the COVID-19 group than in the control group. It contributed the most to the separation between the groups (VIP score >2.0), and according to the ROC analysis result, we concluded that it could be a good biomarker for the diagnosis of COVID-19 disease.

The second, arginine, a semi-essential amino acid, is used as a substrate in several enzymatic reactions and is metabolized to nitric oxide (NO), ornithine, urea, citrulline, agmatine, glutamate, creatine, proline, and polyamines (25, 26). NO and citrulline are formed from arginine in the reaction catalyzed by nitric oxide synthase (NOS). The many physiological functions of NO include neurotransmission, smooth muscle relaxation, inhibition of platelet activation and immune response (27). NO inactivates viruses by modifying the proteins and nucleic acids required for viral replication. It can also reduce in vitro viral replication of various viruses (Herpes virus, Coxsackie virus, Rhino virus, Japanese Encephalitis virus, Hanta virus, Vaccinia virus, Retro virus and the SARS virus). An increase in the level of nasally produced NO is associated with a reduction in cold symptoms. Therefore, NO constitutes a defense mechanism for viruses in the respiratory tract (28). In a recent study, the plasma Arginine level of patients infected with COVID-19 was found to be significantly lower than in healthy controls (29). In the study of Atila et al., it was reported that arginine may be the gold standard in the diagnosis of COVID-19 (21). In addition, another study showed that plasma arginine level is inversely proportional to the severity of COVID-19 (30). In our study, it levels were found to be significantly lower in the COVID-19 group compared to the control group. This decrease supports other studies and may be due to the increase in the use of arginine to increase or compensate the level of NO produced by the body's defense against SARS-CoV-2. According to our results, arginine made a high contribution to the separation of healthy and sick groups (VIP>1.8), and it may be a biomarker with a very high potential to be used in the diagnosis of COVID-19.

Third, proline is an imino acid that is synthesized in humans both through food and endogenously in the body (31). It has important roles in protein synthesis, formation of metabolites (arginine, polyamine and glutamate), oxidative stress reactions, wound healing and body defense (32). Six monogenic congenital defects associated with disruptions in proline metabolism have been identified. It has also been reported to be associated with the neuropsychiatric disorder schizophrenia (33). In the study conducted to evaluate the proline metabolism of people affected by COVID-19, the proline level was found to be significantly lower in the covid group compared to the control group (34). In the metabolomic study by Troisi et al., COVID-19 patients were grouped according to disease severity (asymptomatic, moderately symptomatic, severely symptomatic) and compared with a healthy control group. As a result of the study, proline levels decreased in the control and asymptomatic groups compared to the moderate symptomatic and severe symptomatic groups. The presence of COVID-19 or an increase in the severity of the disease decreased the proline level (35). In the study of Atila et al., COVID-19 patients were divided into four groups (Asymptomatic patients, mildly affected, moderately affected, severely/critically affected) and compared with the healthy control group. As a result of the study, it was reported that the proline level decreased as the severity of the disease increased (21). According to our results, the proline level was found to be significantly lower in the COVID-19 group compared to the control group, which supports other studies. In addition, it has made an important contribution to the separation of sick and healthy individuals and has become the third biomarker with a high potential to be used in the diagnosis of COVID-19 patients.

The relatively small sample size of this study is an important limitation. In addition, metabolomics has been used clinically for the past few years and amino acids have become important metabolites in this field. It is known that factors such as age, gender, lifestyle, diet, measurement techniques and disease severity can affect

the amino acids profile. Therefore, expanding the sample, stratifying patients by disease severity and sex, and analyzing more amino acids (32 amino acids in this study) may help confirm the study results.

## Conclusion

Consequently, COVID-19 viral infection alters and/or affects cellular metabolic pathway, thus causing significant changes in the levels of many amino acids. Identification of changed amino acids can be biomarkers for diagnosis. The spread of metabolic science will make important contributions to a better understanding of the pathophysiology of many diseases, including COVID-19, and to the discovery of diagnostic biomarkers and therapeutic agents.

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***Ethical Approval:*** *The local ethics committee of Harran University Faculty of Medicine (01 June 2020, session 10) approved the study.*

***Author Contributions:*** *Author Contributions: Concept: S.A. Literature Review: S.A., I.K.. Design: S.A., I.K.. Data acquisition: S.A., I.K., E.S.S.. Analysis and interpretation: S.A., I.K.. Writing manuscript: S.A., I.K.. Critical revision of manuscript: S.A., I.K..*

***Conflict of Interest:*** *The authors have no conflicts of interest to declare.*

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**References**

1. El-Shabasy RM, Nayel MA, Taher MM, et al. Three wave changes, new variant strains, and vaccination effect against COVID-19 pandemic. Int J Biol Macromol. 2022; 204:161-168.
2. Covid19.who.int. WHO Coronavirus (COVID-19) Dashboard 2022 [updated 15 May 2022. Available from: https://covid19.who.int/.
3. Pang Z, Zhou G, Chong J, et al. Comprehensive meta-analysis of COVID-19 global metabolomics datasets. Metabolites. 2021;11(1):44.
4. Saccon E, Bandera A, Sciumè M, et al. Distinct Metabolic Profile Associated with a Fatal Outcome in COVID-19 Patients during the Early Epidemic in Italy. Microbiol Spectr. 2021;9(2):e00549-21.
5. Bailey M, Spick MP, Longman K, et al. Untargeted saliva metabolomics reveals COVID-19 severity: Saliva Metabolomics for SARS-COV-2 Prognosis. MedRxiv. 2021.
6. López-Hernández Y, Monárrez-Espino J, Oostdam ASHV, et al. Targeted metabolomics identifies high performing diagnostic and prognostic biomarkers for COVID-19. Sci Rep. 2021;11(1):1-13.
7. Wu YH, Gao SH, Mei J, et al. Jcs: An explainable covid-19 diagnosis system by joint classification and segmentation. IEEE Trans Image Process. 2021;30:3113-26.
8. Poortahmasebi V, Zandi M, Soltani S, et al. Clinical performance of RT-PCR and chest CT scan for COVID-19 diagnosis; a systematic review. Front Emerg Med. 2020;4(2):e57.
9. Fraser DD, Slessarev M, Martin CM, et al. Metabolomics profiling of critically ill coronavirus disease 2019 patients: identification of diagnostic and prognostic biomarkers. Crit Care Explor. 2020;2(10):e0272.
10. Ussher JR, Elmariah S, Gerszten RE, et al. The emerging role of metabolomics in the diagnosis and prognosis of cardiovascular disease. J Am Coll Cardiol. 2016;68(25):2850-70.
11. Doğan HO, Şenol O, Bolat S, et al. Understanding the pathophysiological changes via untargeted metabolomics in COVID-19 patients. J Med Virol. 2021;93(4):2340-49.
12. Pallares-Méndez R, Aguilar-Salinas CA, Cruz-Bautista I, et al. Metabolomics in diabetes, a review. Ann Med. 2016;48(1-2):89-102.
13. Serkova NJ, Standiford TJ, Stringer KA. The emerging field of quantitative blood metabolomics for biomarker discovery in critical illnesses. Am J Respir Crit Care Med. 2011;184(6):647-55.
14. Kume S, Araki Sİ, Ono N, et al. Predictive properties of plasma amino acid profile for cardiovascular disease in patients with type 2 diabetes. PloS one. 2014;9(6):e101219.
15. Liu X, Jin F, Wang C, et al. Targeted metabolomics analysis of serum amino acid profiles in patients with Moyamoya disease. Amino Acids. 2022;54(1):137-46.
16. Leichtle AB, Nuoffer JM, Ceglarek U, et al. Serum amino acid profiles and their alterations in colorectal cancer. Metabolomics. 2012;8(4):643-53.
17. Zhang A, Sun H, Wang X. Serum metabolomics as a novel diagnostic approach for disease: a systematic review. Anal Bioanal Chem. 2012;404(4):1239-45.
18. Asim M, Sathian B, Banerjee I, et al. A contemporary insight of metabolomics approach for COVID-19: Potential for novel therapeutic and diagnostic targets. Nepal J Epidemiol. 2020;10(4):923-27.
19. Song Y, Xu C, Kuroki H, et al. Recent trends in analytical methods for the determination of amino acids in biological samples. J Pharm Biomed Anal. 2018;147:35-49.
20. Karaogul E, Hayoğlu İ, Cicek A, et al. Foods That Improving the Immune System against Covid-19 and Its Infectiousness. International Journal of Current Medical and Biological Sciences. 2021;1(2):58-68.

21. Atila A, Alay H, Yaman ME, et al. The serum amino acid profile in COVID-19. Amino acids. 2021;53(10):1569-88.
22. Wang TJ, Ngo D, Psychogios N, et al. 2-Aminoadipic acid is a biomarker for diabetes risk. J Clin İnvestig. 2013;123(10):4309-17.
23. Estaras M, Ameur FZ, Estévez M, et al. The lysine derivative aminoadipic acid, a biomarker of protein oxidation and diabetes-risk, induces production of reactive oxygen species and impairs trypsin secretion in mouse pancreatic acinar cells. Food Chem Toxicol. 2020;145:111594.
24. Sell DR, Strauch CM, Shen W, et al. 2-aminoadipic acid is a marker of protein carbonyl oxidation in the aging human skin: effects of diabetes, renal failure and sepsis. Biochem J. 2007;404(2):269-77.
25. Adebayo A, Varzideh F, Wilson S, et al. l-Arginine and COVID-19: An Update. Nutrients. 2021;13(11):3951.
26. Wu G, Morris Jr SM. Arginine metabolism: nitric oxide and beyond. Biochem J. 1998;336(1):1-17.
27. Davis KL, Martin E, Turko IV, et al. Novel effects of nitric oxide. Annu Rev Pharmacol Toxicol. 2001;41(1):203-36.
28. Martel J, Ko Y-F, Young JD, et al. Could nasal nitric oxide help to mitigate the severity of COVID-19?. Microbes Infect. 2020;22(4-5):168-71.
29. Rees CA, Rostad CA, Mantus G, et al. Altered amino acid profile in patients with SARS-CoV-2 infection. Proc Natl Acad Sci. 2021;118(25):e2101708118.
30. Sacchi A, Grassi G, Notari S, et al. Expansion of myeloid derived suppressor cells contributes to platelet activation by L-arginine deprivation during SARS-CoV-2 infection. Cells. 2021;10(8):2111.
31. Vettore LA, Westbrook RL, Tennant DA. Proline metabolism and redox; maintaining a balance in health and disease. Amino Acids. 2021;53(12):1779-88.
32. Wu G, Bazer FW, Burghardt RC, et al. Proline and hydroxyproline metabolism: implications for animal and human nutrition. Amino acids. 2011;40(4):1053-63.
33. Hu C-aA, Phang JM, Valle D. Proline metabolism in health and disease. Amino Acids. 2008;35(4):651-2.
34. Ergin Tuncay M, Neselioglu S, Asfuroglu Kalkan E, et al. Modified Proline Metabolism and Prolidase Enzyme in COVID-19. Lab Med. 2022.
35. Troisi J, Cavallo P, Masarone M, et al. Serum metabolomic profile of symptomatic and asymptomatic SARS-CoV-2 infected patients. Res Sq. 2020.