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RESEARCH ARTICLE

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Understanding the pathophysiological changes via untargeted metabolomics in COVID-19 patients

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Abstract

Coronavirus disease 2019 (COVID-19) is an infectious respiratory disease caused by a new strain of the coronavirus. There is limited data on the pathogenesis and the cellular responses of COVID-19. In this study, we aimed to determine the variation of metabolites between healthy control and COVID-19 via the untargeted metabolomics method. Serum samples were obtained from 44 COVID-19 patients and 41 healthy controls. Untargeted metabolomics analyses were performed by the LC/Q-TOF/MS (liquid chromatography quadrupole time-of-flight mass spectrometry) method. Data acquisition, classification, and identification were achieved by the METLIN database and XCMS. Significant differences were determined between patients and healthy controls in terms of purine, glutamine, leukotriene D4 (LTD4), and glutathione metabolisms. Downregulations were determined in *R-S* lactoglutathione and glutamine. Upregulations were detected in hypoxanthine, inosine, and LTD4. Identified metabolites indicate roles for purine, glutamine, LTD4, and glutathione metabolisms in the pathogenesis of the COVID-19. The use of selective leukotriene D4 receptor antagonists, targeting purinergic signaling as a therapeutic approach and glutamine supplementation may decrease the severity and mortality of COVID-19.

KEYWORDS

COVID-19, LC/Q-TOF/MS, leukotriene D4, purinergic signaling, untargeted metabolomics

1 | INTRODUCTION

The new coronavirus, COVID-19, causes severe lower respiratory tract infections in humans. The virus has been the focus of international attention due to the spreading globally.[1] As of July 28, 2020, World Health Organization (WHO) reports that globally there have been 16,114,449 laboratory-confirmed cases of COVID-19 with 646,641 deaths reported.[2] COVID-19 can produce extensive clinical spectrums from the asymptomatic to multiorgan dysfunctions. Lower respiratory tract infection-related symptoms and signs including fever, cough, and/or shortness of breath and pneumonia are the most common clinical findings of the disease.[3] Individuals with immune problems and certain medical conditions such as diabetes, cancer, and cardiopulmonary disease have an increased risk for severe illness from COVID-19.[4] As there is no specific and licensed therapy or vaccine for COVID-19 infection, understanding the disease's pathogenesis is crucially important for the clinical management and epidemiological control of COVID-19 infections.[3] Although the dysregulation of the angiotensin-converting enzyme 2 (ACE2), the alteration of ACE2 receptor expression and cytokine storm are considered important factors in the development of diffuse alveolar damage and severe progression of the disease in COVID-19,[5,6] there is limited data on pathogenesis and the cellular responses of COVID-19 due to the lack of autopsy or histological studies.

The study of metabolomics refers to the comprehensive and quantitative analysis of all metabolites in biological samples. Metabolomics is divided into two approaches, targeted and untargeted. Untargeted metabolomics investigates the comprehensive profiles of all measurable metabolites using high-throughput methods. A wide range of invasive and noninvasive biological samples can be used in metabolomics analysis. Although noninvasive samples, including saliva and urine, can be collected easily, normalization of the metabolome quantities is difficult in these samples. Blood is a primary carrier of metabolites in the body and thus it can provide more information about the metabolome profile changes in the disease state compared to saliva and urine. Therefore, serum and plasma are the most used sample types to better understand the pathophysiological mechanisms of the disease.[7] To date, most studies on COVID-19 have been conducted on routine clinical biochemistry markers, epidemiological data, and clinical findings. Limited numbers of studies have been focused on the molecular changes of the disease. Therefore, it is critical to demonstrate the change of serum metabolome composition to better understand the pathophysiological conditions in COVID-19.

The aim of the present study was to identify the alternation of endogenous metabolites using an untargeted metabolomics approach in serum samples from patients with COVID-19 and healthy controls using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The present work will be beneficial to dissect the underlying mechanisms of COVID-19.

2 | MATERIALS AND METHODS

2.1 | Study design, recruitment, and sample collection

This prospective study was conducted in the Department of Clinical Biochemistry, School of Medicine, Cumhuriyet University. A total of 85 subjects, including 41 COVID-19 patients and 44 healthy controls, were enrolled in the study. The mean ages of patients and controls were 54 ± 19 and 50 ± 15 years, respectively ($p > .05$). The female to male ratios were 1.08 and 0.86 inpatient and control groups, respectively ($p > .05$). The study included patients with clinically diagnosed COVID-19 patients according to the epidemiological history, clinical manifestations, laboratory findings, and real-time polymerase chain reaction (RT-PCR; Bioeksen and Rotor-Gene Q; QIAGEN). RT-PCR detection of COVID-19 was performed from nasopharyngeal and oropharyngeal swabs. Patients admitted to the hospital within 7 days after the onset of symptoms were included in the study. Blood samples were drawn on the first morning after hospitalization. None of the patients required an intensive care unit (ICU) admission. ICU admission was indicated with one of the following conditions: (i) dyspnea and respiratory distress; (ii) respiratory rate >30/min; (iii) partial oxygen pressure/fraction of inspired oxygen <300; (iv) hypotension; (v) acute renal failure, acute increase in liver function tests, confusion, acute bleeding diathesis; (vi) higher troponin levels and arrhythmia; (vii) lactate levels >2 mmol; (viii) dermal pathologies including prolonged capillary refill and cutis marmorata. For the healthy control group, the exclusion criteria included a clinical suspicion of infections or the presence of liver disease, kidney disease, rheumatic disease, malignancy, pregnancy, or smoking. Fasting blood samples were collected in red top tubes (Greiner) with silica to activate clotting of the specimen. After centrifugation (3500 rpm, 15 min, and 4°C), the supernatant was immediately aliquoted and stored at −80°C until analysis. The procedures were approved by the Ethics Committee of Sivas Cumhuriyet University in accordance with the ethical standards established by the institution where the experiments were performed or in accordance with the Helsinki Declaration (Decision number: 2020-04-05).

2.2 | Sample, quality control (QC), and internal standard (IS) preparation

Five hundred microliters of thawed serum samples homogenized on a vortex mixer, mixed with 1000 μl of acetonitrile, and incubated. The sample was vortexed and then stored in a refrigerator for 10 min then centrifuged for 5 min at 16,900*g*. Five hundred microliters of supernatant was transferred into a clean microtube. Glycine d5 was selected as the IS to improve data quality and check the autonomous integration success of the software. Besides this, to guarantee mass accuracy, a reference solution was directly infused into the source, enabling continuous internal calibration during analysis and ensuring accuracy and reproducibility. For that purpose, purine (*m/z* 121.0508) and hexakis phosphazinen (*m/z* 922.0097) signals were used. The reproducibility standard deviation of each IS was under 20%. The blank sample was prepared with a mobile phase mixture containing only IS. In addition to this, QC samples were prepared according to the above procedure after 5 μl of each sample was collected. The blank sample was injected to observe any interference due to instrument and media. QC samples were injected into an LC/Q-TOF/MS system to remove false positives from the system.

2.3 | Liquid chromatography–mass spectrometry (LC–MS) analysis

The LC/Q-TOF/MS system consisted of an Agilent 1290 Infinity LC system coupled with Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Agilent Technologies, Inc.) and a ZORBAX RRHD Eclipse Plus C18, 95 Å, 2.1 × 100 mm, 1.8 μm (Agilent Technologies, Inc.). The mobile phase system consisted of % 0.1 formic acid in water (A) and acetonitrile (B) using a gradient elution as follows: A mobile phase flow rate of 0.4 ml/min was employed for gradient elution at a total of 12-min run time as follows: 0–0.5 min, 2% B; 0.5–4 min, 20% B; 4–8 min, 50% B; 4–6 min, 95% B; 8–9 min, 95% B; 9–9.25 min, 2% B and 9.25–12 min, 2% B for equilibration of the column. The column temperature was set at 55°C

during the analysis. The injection volume was 4.0 µl. A positive ion scan mode at 3.5-kV capillary voltage was applied during the study. Mass scanning was ranged from 50 to 1000 *m/z*. The ion source was Electronspray Ionization (ESI). The MS absorbance threshold was set at 200. The instrument acquired data by optimized parameters as drying gas temperature, 350°C; drying gas flow, 11 L/min; nebulizer, 40 psi; sheath gas temperature, 350°C; sheath gas flow, 11 L/min.

3 | RESULTS

All samples and QC aliquots were analyzed via Q-TOF MS/MS. The total ion chromatogram of the study was monitored in Figure 1. Such complex chromatograms mostly include thousands of peaks having huge intensity alterations across the spectrum. Due to that reason, several algorithms were improved for peak detection. In this study, the XCMS centWave algorithm was selected for peak detection. All samples were imported into the XCMS R package, and peak detection was maintained. The Obiwarp algorithm of the mentioned package was used for peak alignment. The retention time of each chromatogram was optimized by this approach. Then peak grouping step was achieved to obtain the unique retention time and *m/z* value which called "feature" for the analysis. After all, an excel sheet was generated for all samples, including retention time, unique *m/z* values, and intensity for each feature. This excel sheet was imported into MATLAB for chemometric analysis. Features that have low reproducibility in QCs and detected in blanks were removed. Quality Control—Support Vector Regression (QC-SVR) correction was also maintained. After cleaning the data, the classification was performed by importing the data into PLS Toolbox 8.0. Partial Least Square—Discriminant Analysis (PLS-DA) was implemented for each group. Three latent variables were selected to explain the model. LV1–LV2–LV3 score plots were monitored in Figure 2, which exhibits the alteration of metabolites between each group. Regarding Figure 3, groups were certainly separated. Receiver operating characteristic curve (ROC) analyses were also made to evaluate the selectivity and sensitivity of the model. The specificity versus selectivity graph shows that the model is not overfitted, and chance correlation is not possible. Fifty iterations proceeded for the proposed model. The cross-validation data set had an acceptable area under the curve (AUC) value (0.9989) in accordance with Figure 4. The regression coefficient for the model and cross-validation data set was 0.997 and 0.906, respectively. AUC values were calculated for ROC curves, which were found as 1.00 and 0.9989 for calibration and cross-validation data set.

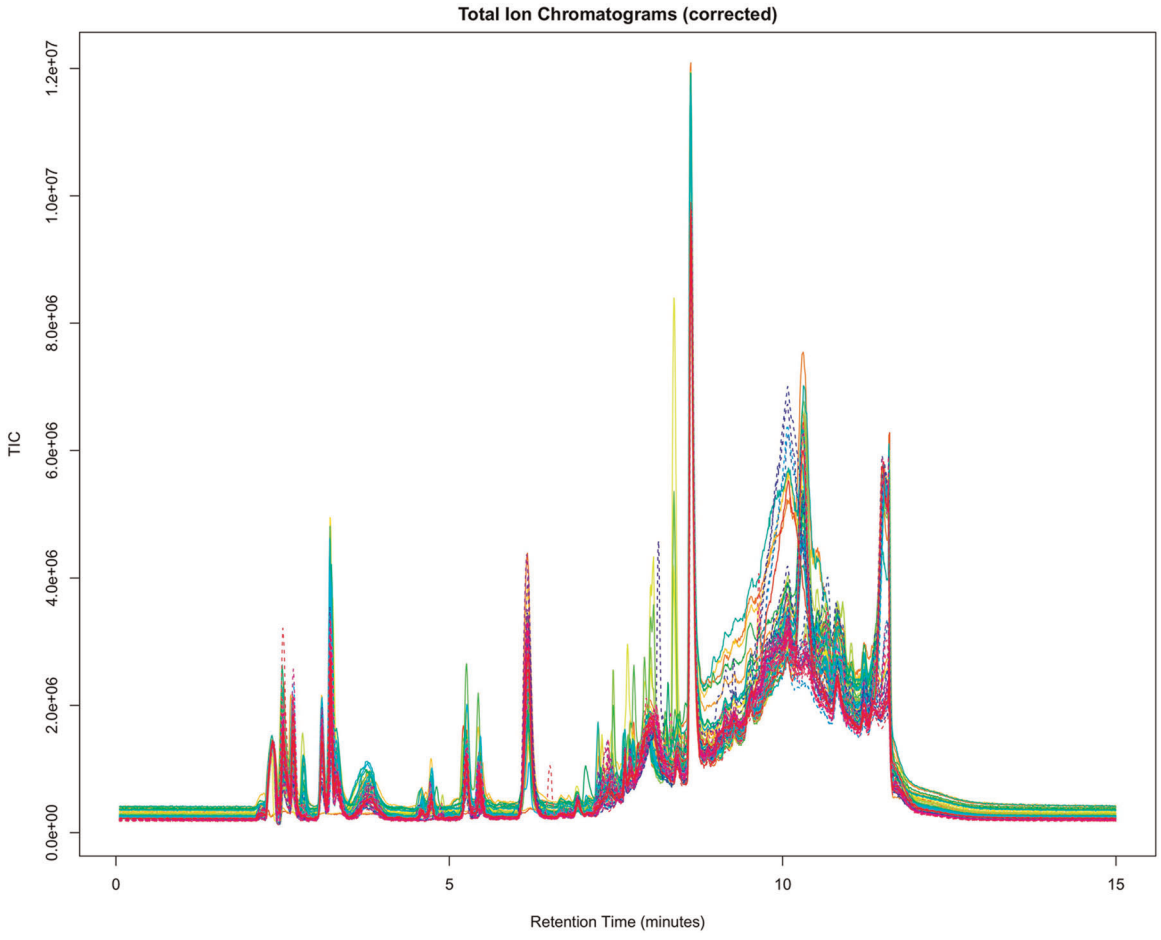

FIGURE 1 Total ion chromatogram of each sample via Q-TOF MS/MS. Q-TOF MS/MS, quadrupole time-of-flight tandem mass spectrometry

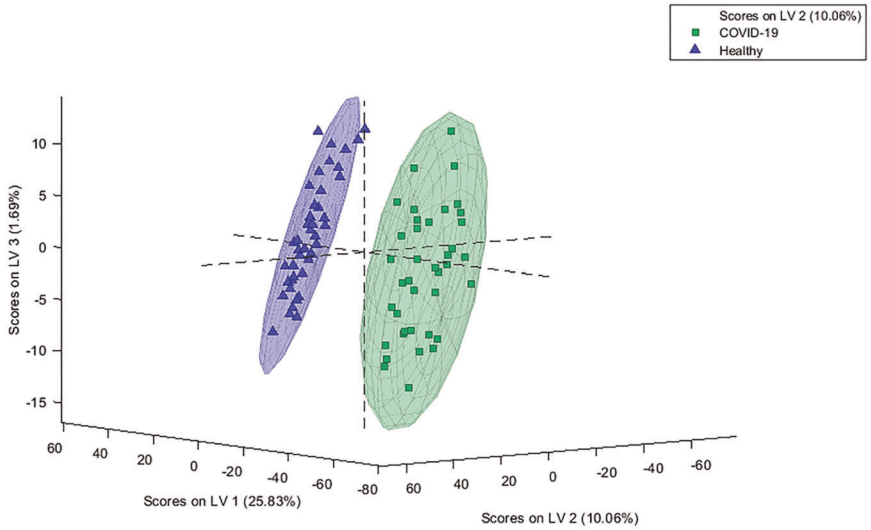

FIGURE 2 Score plot of latent variables in accordance with PLS-DA model. PLS-DA, partial least square—discriminant analysis

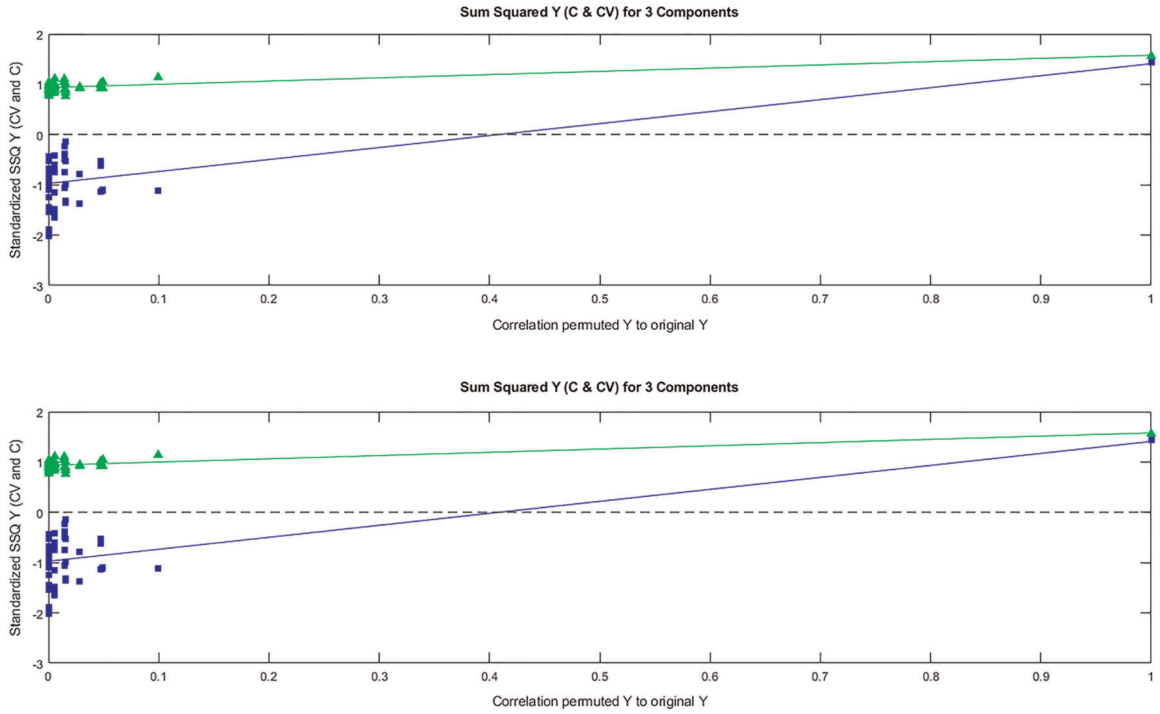

FIGURE 3 Permutation test for the test for PLS-DA model. PLS-DA, partial least square—discriminant analysis

All ROC curve studies and permutation tests have shown that method is not over-fitted and can perfectly classify the COVID-19 group compared to healthy volunteers (Figure 4). Variables in projection results suggest that over 300 features were statistically significant for such an alteration (Figure 5). Features were identified by the parameters such as positive ESI mode and using a 10 ppm tolerance, mummichog (default *p* value cutoff settings), and in accordance with Kyoto Encyclopedia of Genes and Genomes (KEGG) database for human research. Metaboanalyst 4.0 software was used for pathway analysis. According to the data, 26 different pathways were significantly affected by the COVID-19 cases, which are briefly given in Table 1 and the pathway map is given in Figure 6.

The major pathways varied in the patient versus control group were given in Figure 7. Metabolites significantly differentiating serum profiles of COVID-19 patients from healthy controls were given below in Table 2.

4 | DISCUSSION

Prominent differences in purine, leukotriene D4 (LTD4), glutathione, and glutamine metabolisms have been observed in COVID-19 patients. Downregulations were observed in *R-S* lactoglutathione and glutamine. Upregulations were detected in hypoxanthine,

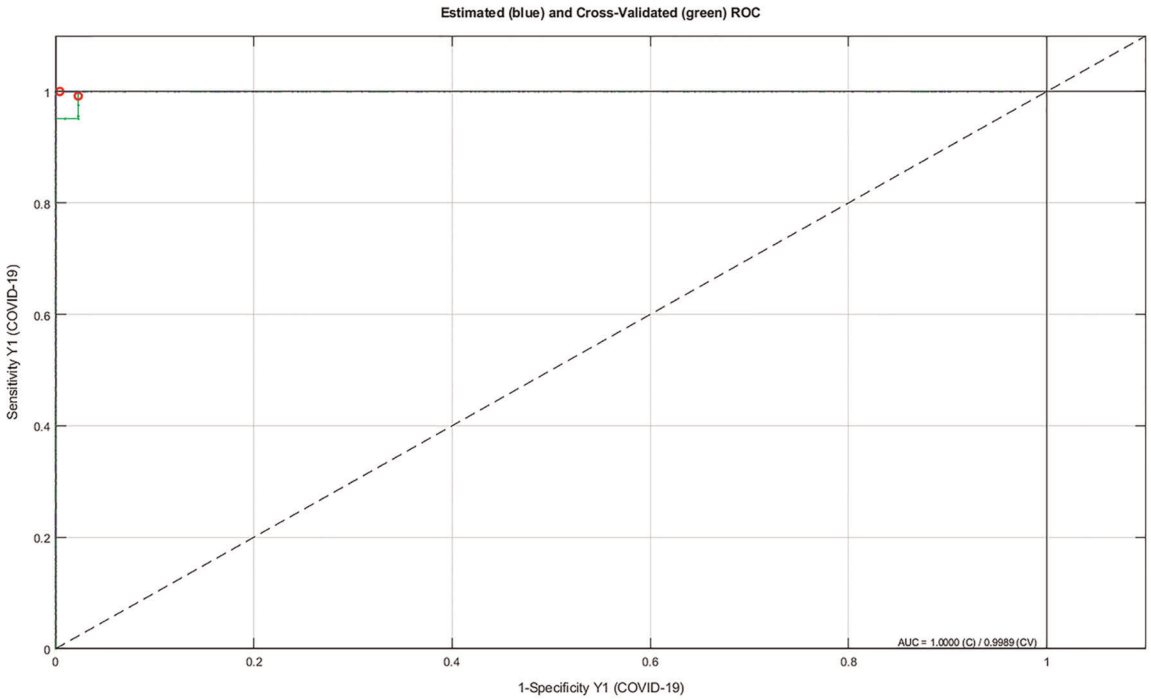

FIGURE 4 ROC curve for model and cross-validation data sets. COVID-19, coronavirus disease 2019; ROC, receiver operating characteristic curve

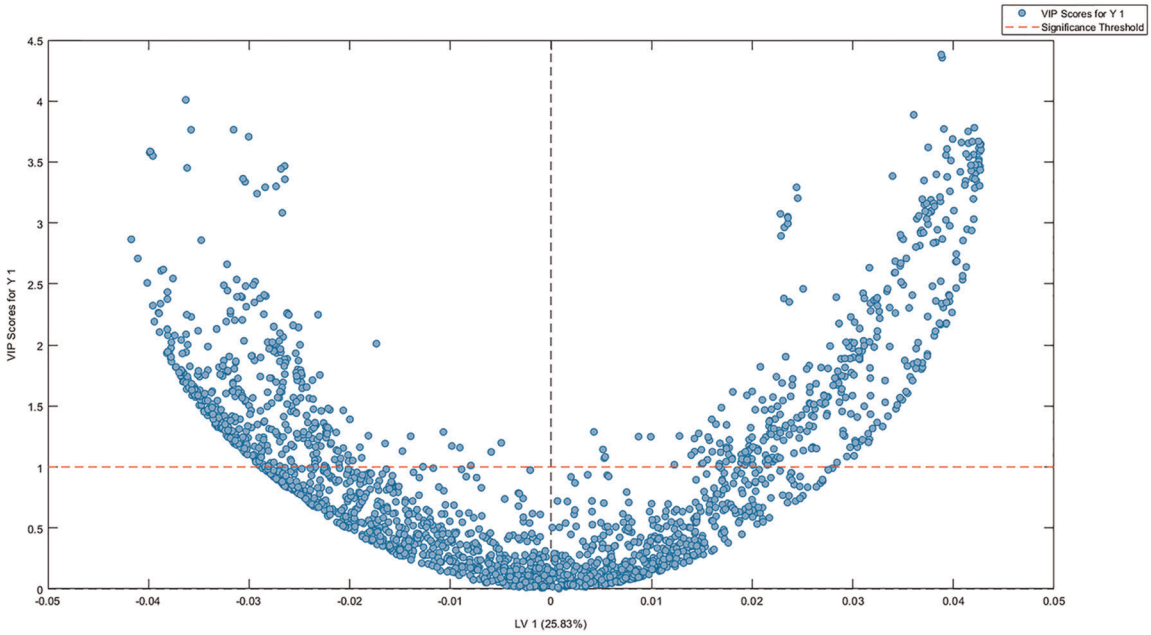

FIGURE 5 Variables in projection (VIP) scores for the PLS-DA model. PLS-DA, partial least square—discriminant analysis

inosine, and leukotriene D4. The possible reasons for the observed changes in COVID-19 patients are explained and compared with previous studies.

In the present study, upregulated hypoxanthine and inosine statuses, which are related to purine metabolism, are found in COVID-19 patients compared to healthy controls. Wu et al.[8] found significantly changed purine metabolism in the untargeted metabolomics analysis of COVID-19 patients. Our finding is in accordance with the study. Besides this, in a previous untargeted metabolomics study performed on the murine model of influenza pneumonia, higher hypoxanthine, and inosine expressions have been detected in lung and bronchoalveolar lavage fluid.[9] In line with

TABLE 1 Affected pathways in coronavirus disease 2019 (COVID-19) patients compared to healthy controls

Pathways	Total (metabolites)	Annotated	Significant
Purine metabolism	13	13	9
Leukotriene metabolism	4	4	1
Histidine metabolism	5	5	3
Glutamine and glutamate metabolism	5	5	3
Glutathione metabolism	5	5	3
Alanine, aspartate and glutamate metabolism	3	3	2
Glyoxylate and dicarboxylate metabolism	3	3	2
Nitrogen metabolism	3	3	2
Pentose phosphate pathway	1	1	1
Pentose and glucuronate interconversions	1	1	1
Pyrimidine metabolism	4	4	2
Taurine and hypotaurine metabolism	1	1	1
Phosphonate and phosphinate metabolism	1	1	1
Glycerophospholipid metabolism	1	1	1
Ether lipid metabolism	1	1	1
Arginine biosynthesis	9	9	3
Arginine and ornithine metabolism	2	2	1
Butanoate metabolism	2	2	1
Arginine and proline metabolism	10	10	3
Primary bile acid biosynthesis	3	3	1
Inositol phosphate metabolism	3	3	1
Phosphatidylinositol signaling system	3	3	1
Drug metabolism—cytochrome P450	4	4	1
Lysine degradation	5	5	1
Porphyrin and chlorophyll metabolism	5	5	1
Aminoacyl-tRNA biosynthesis	16	16	2

this literature information and our finding, we believe that there is an association between the pathophysiology of COVID-19 and altered purine metabolism. Hypoxanthine and inosine are the intermediate metabolites of adenosine breakdown. Adenosine is a purine base and required for adenosine triphosphate (ATP) synthesis. The disease mainly affects the lungs therefore, respiration-related symptoms, including hypoxia, are the main clinical findings.[10] Inflammation and hypoxia induce the release of ATP from intracellular stores to extracellular space, and then it is converted to adenosine monophosphate (AMP), which is then metabolized to adenosine and three phosphates. Finally, adenosine is converted to inosine and hypoxanthine by activating adenosine deaminase and purine nucleoside phosphorylase, respectively.[11–14] Hypoxanthine concentration in blood is found to be a sensitive parameter of hypoxia.[13,15] Therefore, it is thought that upregulations of hypoxanthine and inosine are related to increased breakdown of ATP due to the hypoxic condition in COVID-19 patients.

Purinergic signaling has an essential regulatory role in acute and chronic lung inflammations. It is mainly driven by ATP, AMP, adenosine, and inosine.[11,16] Protective roles of adenosine signaling via the activation of adenosine 2A (A2AR) and 2B (A2BAR) receptors have been shown in acute lung inflammation.[17,18] A2BAR receptors contribute to adaptation to inflammation, ischemia, and hypoxia. It has also been reported that inosine has an anti-inflammatory effect against acute lung injury caused by cytokines.[16] Accordingly, it is thought that purinergic signaling has a regulatory role in COVID-19 against inflammation and hypoxic condition. The inhibition of the adenosine and inosine breakdown to hypoxanthine by the inhibition of adenosine deaminase and purine nucleoside phosphorylase can be effective in attenuating severe COVID-19 disease.

In this study, statistically significant changes have been detected in glutathione metabolism in patients compared to healthy controls. In a recent study, affected glutathione metabolism has been observed in the untargeted metabolomics analyses of COVID-19 patients.[8]

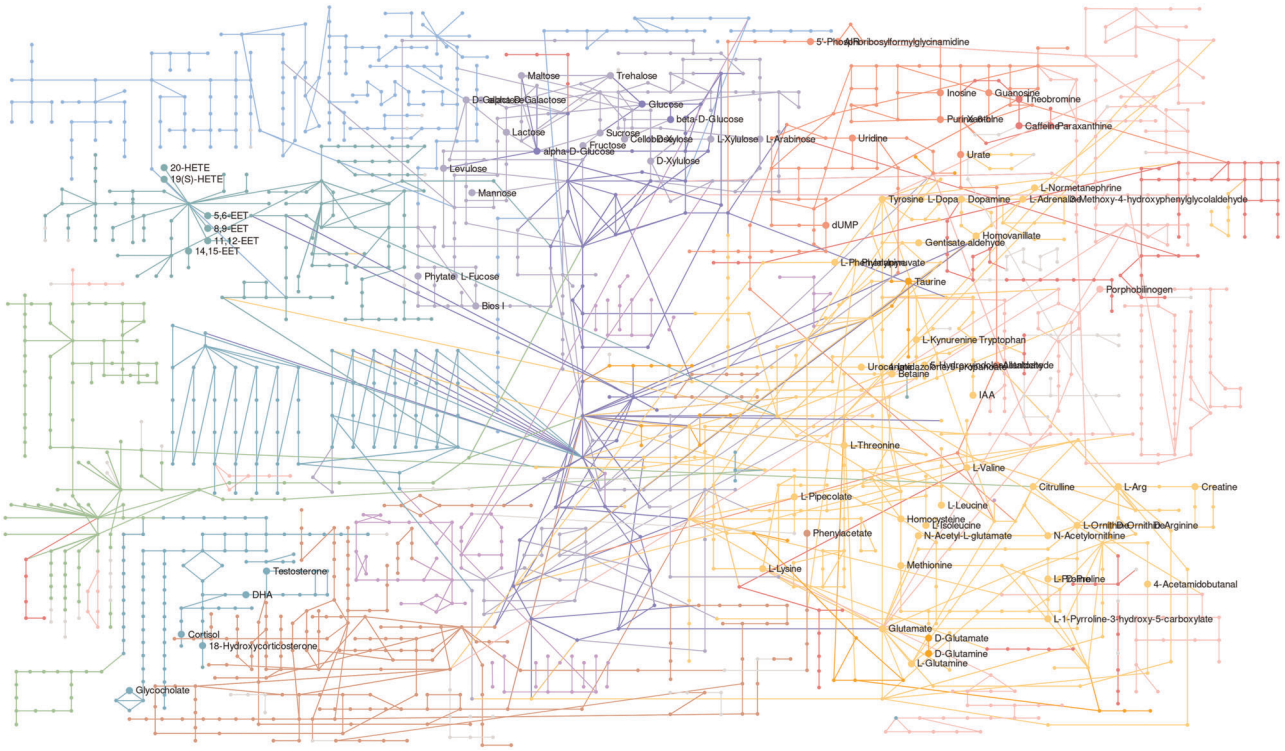

FIGURE 6 Pathway map for the significantly altered metabolites in COVID-19 patients. COVID-19, coronavirus disease 2019

Our finding is in line with this study. Downregulations were detected in (*R*)-*S*-lactoylglutathione and L-glutamate. *R*-*S*-lactoylglutathione is an intermediate metabolite of pyruvate metabolism and can be converted into glutathione by activating hydroxyacylglutathione hydrolase.[19] Glutamate is required for the synthesis of glutathione by two cytoplasmic sequential ATP-dependent reactions.[20] Glutathione metabolism has an essential role in maintaining matrix redox homeostasis as an antioxidant.[19,21]

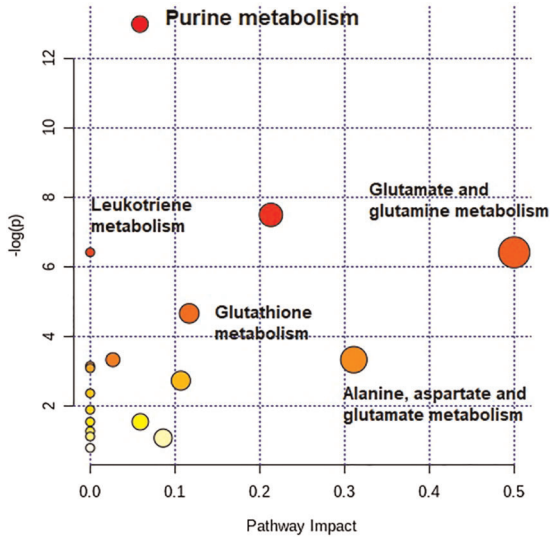

FIGURE 7 Pathway analysis generated by MetaboAnalyst online: the major pathways varied in the patient versus control group

Moreover, it also has crucial roles in regulating cell proliferation, apoptosis, immune function, and fibrogenesis.[22] Increased oxidative stress and disrupted antioxidant defense has been shown in experimental models of severe acute respiratory syndrome coronavirus (SARS-CoV).[23,24] In view of the findings mentioned above, it is thought that disrupted balance between oxidant and antioxidant metabolism due to the glutathione depletion may be related to severity and mortality risk in patients with COVID-19. Additionally, (*R*)-*S*-lactoylglutathione can be a potential biomarker for evaluating antioxidant metabolism in COVID-19.

In our study, upregulation in patients is also observed in leukotriene D4 (LTD4), which is an inflammatory lipid mediator derived from arachidonic acid.[25] Leukotrienes have pivotal roles in regulating acute and chronic inflammation as important chemoattractants for neutrophils and lymphocyte subgroups, which are mainly observed inflammatory cells in the airways of COVID-19 patients. Besides this, it provokes macrophage activation and pro-inflammatory cytokine secretion.[26] These effects of leukotrienes cause bronchoconstriction, edema, vasoconstriction, vascular leakage, and mucus secretion in the airways.[27] Increased vascular leakage and uncontrolled inflammation have been associated with alveolar damage in COVID-19 patients.[26] Accordingly, we think that increased expressions of LTD4 have a role in the pathophysiology of COVID-19. Glutathione is an essential substance in the formation of LTD4. LTA4 is conjugated with glutathione with activation of leukotriene C4 synthase to produce leukotriene C4 that is a precursor substance of LTD4.[28,29] As we indicated in the previous paragraph, we found downregulation of glutathione in patients. Therefore, we speculate that increase

TABLE 2 List of the significantly different metabolites identified in patient versus control group

KEGG ID	Compound	Exact mass	Measured mass	Λ mass error (ppm)	Fold	Retention time	*p* value	Regulation
C01879	5-Oxoproline	129.0426	129.0436	7.75	2.10	2.46	.00782	Up
C00515	Ornithine	132.0899	132.0867	6.06	1.80	2.25	.0492	Up
C00262	Hypoxanthine	136.0385	136.0355	7.35	6.10	3.24	.00240	Up
C00785	Urocanate	138.0429	138.0437	5.79	1.90	3.14	.04512	Up
C00064	L-Glutamine	146.0691	146.0702	7.53	2.00	6.93	.00017	Down
C00025	L-Glutamate	147.0532	147.0545	8.84	2.00	2.66	.047	Down
C00121	D-Ribose	150.1299	150.1313	9.33	3.00	5.14	.0399	Up
C03680	4-Imidazolone-5-propionic acid	156.0535	156.0548	8.33	6.40	8.26	.04528	Down
C00499	Allantoic acid	176.0546	176.0532	7.95	1.60	9.80	.0204	Up
C12248	2-Oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline	202.0338	202.0350	5.94	1.70	2.37	.02137	Up
C00294	Inosine	268.0808	268.0820	4.48	6.70	3.44	.0362	Up
C00387	Guanosine	283.0917	283.0941	8.48	1.50	6.18	.04683	Down
C03838	5′-Phosphoribosylglycinamide	286.0566	286.0540	9.09	2.10	9.02	.00294	Down
C16609	Didemethylcitalopram	296.1325	296.1345	6.75	1.80	9.59	.0315	Up
C00365	Deoxyuridylic acid	308.0410	308.0402	2.60	1.70	7.58	.00033	Up
C03451	*R-S* lactoglutathione	379.1049	379.1071	5.80	3.07	8.80	.00213	Down
C05951	Leukotriene D4	496.2607	496.2559	9.67	1.30	7.85	.0200	Up

Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

production of LTD4 causes excessive use of glutathione, and this condition contributes to glutathione depletion in patients. Drug-targeted LTD4 receptors have been widely used in asthma to reduce bronchoconstriction and increased pulmonary vascular edema. Although the relationship was established in small-scale or non-randomized, placebo-controlled trials, leukotrienes were also associated with viral bronchiolitis.[25] The LTD4 receptor antagonist has an anti-apoptotic effect. Besides this, these drugs can decrease oxidative stress and cytokine production.[30,31] Cytokine storm has been related to mortality and complications including, respiratory failure in COVID-19 patients.[32] NF-κB has an important role in the expression of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. It is shown that certain leukotriene receptor antagonists could inhibit NF-κB activation.[31] Finally, in two perspective studies, it is hypothesized that cysteinyl leukotriene receptor antagonist could be used for treatment in COVID-19 patients because of its anti-inflammatory effects.[26,33] Our findings support this hypothesis. Thus, selective leukotriene D4 receptor antagonists may limit the complications related to uncontrolled immune system activation in lung tissues of COVID-19 patients.

Our results reveal downregulation of glutamine in COVID-19 patients compared to healthy controls. In a recent study, significantly decreased glutamine expressions have been detected in the untargeted metabolomics analysis of COVID-19 patients.[34] Our finding is in accordance with this study. Glutamine is the most abundant amino acid in the body and has antioxidant and anti-inflammatory effects.[35] Lungs contribute to maintaining glutamine homeostasis in human as well as skeletal muscle. Glutamine depletion has been associated with different pulmonary diseases including asthma, bronchopulmonary dysplasia, chronic obstructive pulmonary disease, acute respiratory distress syndrome, and lung fibrosis.[36] It has been demonstrated that exogenous glutamine administration significantly reduces mortality and inflammatory lung injury in animal models of acute respiratory distress syndrome by the regulatory roles on cytokine release, neutrophil infiltration, macrophage function, and apoptosis.[37,38] The mortality rate of COVID-19 related to comorbidities such as hypertension, hyperlipidemia, glucose intolerance, obesity, and diabetes are at a higher risk of developing severe disease.[4] It has been shown that glutamine attenuates numerous risk factors, such as obesity, hypertension, diabetes, and hyperlipidemia.[39] Decreased blood glutamine levels at ICU admission have been considered as an independent risk factor for post-ICU mortality.[40] Cengiz et al.[41] stated that L-glutamine supplementation led to a shortened hospital stay and less need for ICU in COVID-19 patients. In line with this literature information and our findings, it is proposed that glutamine has a role in the regulation of lung inflammation and repair in COVID-19. Additionally, our results also confirm that L-glutamine administration can be a novel target therapy for COVID-19 to decrease comorbidities-related mortality and inflammatory lung injury.

We are aware that our research has a limitation. We did not perform LC-QTOF-MS analysis in the negative mode. For this reason, negative polarity metabolomes could not be detected. The findings of the study have to be seen in light of this limitation.

5 | CONCLUSION

COVID-19 has been seen in 216 countries worldwide and is now accepted as a pandemic. There is limited data on the pathogenesis and the cellular responses of COVID-19. Besides this, there is no specific and proven vaccine or antiviral therapy. Due to these reasons, understanding its mechanism has vital importance. In this study, COVID-19 was analyzed by using metabolomics. Changed purine, glutamine, glutathione, and LTD4 metabolism were detected in patients compared to healthy controls. Glutamine and (*R*)-*S*-lactoylglutathione depletion, the activation of LTD4 pathway, and purinergic signaling may have a role in the regulation of lung inflammation in COVID-19. Disrupted oxidant and antioxidant balance may play a part in the formation of lung damage in COVID-19 patients. (*R*)-*S*-lactoylglutathione may be a useful indicator of antioxidant status in COVID-19. The results of the present study also suggest that the use of selective leukotriene D4 receptor antagonists, targeting purinergic signaling as a therapeutic approach and, glutamine supplementation may decrease the severity and mortality of COVID-19.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHORS CONTRIBUTIONS

Halef O. Doğan: conceptualization, methodology, resources, and writing—original draft. **Onur Şenol**: methodology, data curation, and visualization. **Serkan Bolat**: investigation and visualization. **Şeyma N. Yıldız**: methodology and investigation. **Seyit A. Büyüktuna**: investigation. **Rağıp Sarıismailoğlu**: investigation. **Kübra Doğan**: investigation. **Mürşit Hasbek**: investigation. **Süleyman N. Hekim**: writing—review and editing.

DATA AVAILABILITY STATEMENT

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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