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Serum HMGB1, DKK1, and ACE2 as a Function of Lung Injuries in COVID-19 Patients

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ABSTRACT

There is a need for a biomarker for lung injury in COVID-19 patients. In the present study, an attempt was carried out to examine the role of Dickkopf-related protein 1 (DKK1), High-mobility group box 1 protein (HMGB1), angiotensin-converting enzyme 2 (ACE2) as a function for the lung abnormalities in CT-scan (LACTS). To perform the goals, DKK1, HMGB1, and ACE2 were measured in patients and controls using the ELISA technique. In contrast, other parameters were measured spectrophotometrically. The results showed decreased SpO_2 and albumin and an increase in the serum biochemical parameters (glucose, urea, creatinine, D-dimer, ACE2, DKK1, and HMGB1) in COVID-19 patients compared with the control group. In COVID-19 patients, the percentages of the lung abnormalities in CT-scan% are 40.67±11.84. The results showed that those patients with LACTS patients are slightly older and have lower SpO_2 than the patients without the LACTS group. ACE2 shows a significant correlation with SpO_2 ($\rho = 0.336$, $p<0.01$) and a negative correlation with albumin ($\rho = -0.197$, $p<0.05$). Other parameters showed no significant correlation with the measured biomarkers. In conclusion, COVID-19 patients have higher ACE, DKK1, and HMGB1 indicating the involvement of the pathways of these biomarkers in the disease progression including lung injury.

1. Introduction

The world faces a grave public health hazard in the aftermath of the third coronavirus pandemic, caused by a new coronavirus (severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an infection with which can result in Covid-19 (Englisch *et al.* 2021). COVID-19 patients often have severe symptoms such as acute respiratory distress syndrome (ARDS), inflammation, hypercoagulation, and thrombosis due to potential cytokine storm (Tan *et al.* 2021). The range of SARS-CoV-2 infections in a clinical context varies from asymptomatic to moderate upper respiratory tract disorders to severe respiratory pneumonia and even death (Zhou *et al.* 2020).

After the interaction of viral Spike proteins S with ACE2 receptors, the COVID-19 virus penetrates in the lungs (Amini Pouya *et al.* 2020). The virus may thus produce histopathological lesions similar to those found in other types of ARDS (Merdji *et al.* 2021). Chest imaging, particularly computed tomography scan (CT-scan), is critical for diagnosing, treating, and following COVID-19 infected patients (Fang 2020). The presence of ground-glass opacity (GGO) in COVID-19, for example, implies severe or chronic inflammation in the lungs and more severe symptoms such as bronchiolitis and pneumonia in both lungs and lung fibrosis (Sadhukhan *et al.* 2020). These areas of a lung infection can recruit various immune cells, leading to a pro-inflammatory reaction, including high IL-6 levels (Sadhukhan *et al.* 2020). These findings showed that lung abnormalities may lead to reduced oxygenation as a result of the chest CT scan (Sadhukhan *et al.* 2020).

High mobility group box 1 (HMGB1) is a prototypical alarmin activating innate immunity and serves as a dual alarmin function (Andersson *et al.* 2018). Extracellular HMGB1 triggers inflammation and recruits leukocytes to the site of tissue damage (Venereau *et al.* 2013) and is involved in inflammasome activation and autophagy (Tsung *et*

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al. 2014). The DAMP family was identified by HMGB1 as one of the earliest members. Inflammatory signals are generated by HMGB1 in several ways, by either direct activation of the toll-like receiver (TLR) 4 or by forming complexes with extracellular DNA, RNA, and other DAMP complexes (PAMPs) (Yang *et al.* 2015). Several chronic inflammatory or autoimmune conditions are implicated in HMGB1 protein. For example, influenza-infected individuals who develop severe pneumonia have increased levels of HMGB1 in their bloodstream (Hou *et al.* 2014). Disease severity is associated with higher pro-inflammatory cytokines suggestive of a cytokine storm in the most severe COVID-19 patients (Zhou *et al.* 2020). HMGB1 levels were strongly associated with plasma IL-10 and IFN-, both of which are well-known indicators of COVID-19 development (Chi *et al.* 2020).

Dickkopf-related protein 1 (DKK1) is a soluble protein that acts as an antagonist of the Wnt/β-catenin signalling pathway (Mazon *et al.* 2018). By inhibiting Wnt/-catenin signalling and inducing cellular senescence, Dkk1 acts as a secreted growth inhibitor (Lyros *et al.* 2014). DKK1 was altered in many other illnesses with various methods that commonly end with the inflammatory pathway. The expression of serum DKK1 was elevated in individuals with lung cancer (Sun *et al.* 2020). Given that DKK1 is a well-known direct-catenin target gene, the reported stimulation of canonical Wnt/-catenin signalling might justify DKK1 overexpression (Lyros *et al.* 2019).

SARS-CoV-2 uses the angiotensin-converting enzyme-2 (ACE2) as receptors when it enters the human body (Sinanović *et al.* 2020). Given the importance of the ACE2 receptor in the entrance of SARS-CoV-2 to enter host cells, the question of the involvement of ACE inhibitors and angiotensin-receptor blockers (ARBs) in the pathogenesis of COVID-19 emerges as a highly complex relationship that is still highly contentious. On the one hand, it has been discovered in animal models that ACE inhibitors and ARBs can enhance the expression of ACE2 receptors (Wang *et al.* 2016), which presumably increases SARS-capacity CoV-2's to penetrate cells. However, the research on this is inconclusive, and it is possible that it is tissue-dependent, dose-dependent, and varies amongst ARBs (Wang *et al.* 2016).

In the present study, an attempt was carried out to explore the role of some less-studied molecules in COVID-19 patients, namely, DKK1, HMGB1 on the level of COVID-19 receptor (ACE2). The effect of other parameters, including the lung abnormalities in the CT-scan and the saturation oxygen percentage (SpO_2), on the levels of these parameters were examined.

2. Materials and Methods

2.1. Subjects

The study has been authorized by the University of Kufa's Institutional Review Board (IRB) (177/2021). Before participating in this study, all controls and patients provided written informed permission. The study was done in compliance with Iraqi and international ethical and privacy regulations and the World Medical Association's Declaration of Helsinki.

Sixty patients with confirmed SARS-CoV-2 infection and thirty healthy controls were enrolled in this research. Between September and November 2020, patients were recruited at the Al-Amal Specialized Hospital for Communicable Diseases, Al-Hakeem General Hospital, Al-Shifa Health Center, and Al-Amal Specialized Hospital for Infectious Diseases in Najaf Governorate-Iraq between January 2020 till February 2021. SARS-CoV-2 infection was diagnosed based on positive reverse transcription real-time polymerase chain reaction (rRT-PCR) results for COVID-19 nucleic acids and positive IgM antibodies to SARS-CoV-2, as well as symptoms of acute infectious disease such as fever, fatigue, breathing difficulties, coughing, and loss of smell and taste. Patients with premorbid medical conditions such as diabetes, liver disease, chronic renal disease, neurodegenerative and neurologic diseases such as multiple sclerosis, Parkinson's, or Alzheimer's disease were excluded.

We examined chest computed tomography (CT) images to detect lung abnormalities in CT-scan (LACTS), which included GGOs, pulmonary densification regions consistent with residual lesions, pneumonic consolidation, and crazy-paving patterns (Kwee and Kwee 2020). We divided the patients into those who received (COVID+LACTS) and those who did not get (COVID-LACTS). The patients were then split into negative-IgG and positive-IgG subgroups based on their IgG findings to investigate the differences in the detected biomarkers between these subgroups. Additionally, 30 healthy controls were selected who were age and sex-matched to the patient groups. None of the controls had any systemic illness. However, as a public health measure to boost their resistance to COVID-19 infection, some healthy controls received zinc and vitamins C and D.

2.1.1. Measurements

RT-PCR tests were performed on the patient during hospitalization using the Lyra® Direct SARS-CoV-2 Assay

kits (Quidel Corporation, CA, USA) and the Applied Biosystems® QuantStudioTM 5 Real-Time PCR System (Thermo Fisher Scientific; Life Technologies Holdings Pte Ltd., Marsiling Industrial Estate, Singapore). The Lyra® Direct SARS-CoV-2 assay kit is a quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay for detecting human coronavirus SARS-CoV-2 from viral RNA isolated from nasal, nasopharyngeal, or oropharyngeal swab specimens.

Fasting blood samples were collected in the early morning immediately following participation in the research. Five millilitres of venous blood were taken and placed in clean, simple tubes. After ten minutes, the clotted blood samples were centrifuged at 3,000 rpm for five minutes, and then the serum was separated and transferred to three fresh Eppendorf tubes until the assay. Samples that had been hemolyzed were discarded. The serum titer is calculated as the reciprocal of the highest dilution eliciting a positive reaction multiplied by the concentration of positive control. The following formula is used to estimate the CRP concentration in a patient sample: 6 times the CRP Titer equals mg/l. sACE2, DKK1, and HMGB1 levels in serum were determined using ELISA kits provided by Melsin Medical Co, Jilin, China. All kits utilized the sandwich method and demonstrated an inter-assay CV of less than 12%. Serum albumin, urea, creatinine, and glucose concentrations were determined spectrophotometrically using Biolabo® kits, Maizy, France. The processes were carried out exactly as specified by the manufacturer. To identify IgG and IgM in the serum of all participants, a qualitative ACON® COVID-19 IgG/IgM fast test was utilized. The kits have a 99.1 per cent sensitivity and a 98.2 per cent specificity.

2.1.2. Statistical Analysis

The analysis of variance (ANOVA) was employed to analyze continuous variables, while the analysis of contingency tables (χ^2-test) was utilized to evaluate nominal variables. Spearman's rank-order correlation coefficients determined the connections between biomarkers and clinical and cognitive scores. To identify connections between diagnoses and biomarkers, we used multivariate general linear models (GLMs). To compute the influence, partial eta-squared values were used. We used multiple regression analysis to find significant biomarkers while accounting for age, gender, and education effects. Statistical significance was found using a p-value of 0.05. All statistical analyses were done using IBM SPSS v25, 2017.

3. Results

3.1. Demographic, Clinical, and Biochemical Biomarkers between COVID-19 Patients and Controls

The results of demographic and clinical data healthy controls (HC) and COVID-19 patients are presented in Table 1. There is no significant difference ($p>0.05$) between COVID-19 patients and the healthy control group in age, sex, BMI, and DBP, as shown in Table 1. Decreased SpO_2 and albumin ($p<0.05$) were also seen in COVID-19 patients compared with the healthy groups. In patients, there is a substantial rise in body temperature ($p<0.001$) and SBP ($p = 0.002$). In COVID-19 patients, the percentages of the LACTS% are 40.67±11.84. There is a significant increase in the serum biochemical parameters (glucose, urea, creatinine, D-dimer, ACE2, DKK1, and HMGB1) in patients compared with the control group.

3.2. Comparison between COVID-19 Patients with and without LACTS

The biomarkers in COVID-19 patients were divided into those with lung abnormalities, the chest CT scan (+LACTS) and without LACTS (-LACTS). The findings are presented in Table 2. The results showed that that +LACTS patients are slightly older ($p<0.042$) and have lower SpO_2 ($p = 0.019$) than the -LACTS group. Patients with +LACTS also have a significant elevation in the serum levels of urea ($p<0.001$), creatinine ($p = 0.008$), glucose ($p = 0.041$), and HMGB1 ($p<0.001$). While other parameters showed no significant difference between -LACTS and +LACTS groups.

3.3. Correlation between ACE2, DKK1, and HMGB1 with Demographic and Clinical Data in COVID-19 Patients

Table 3 shows the correlation between ACE2, DKK1, and HMGB1 with demographic and clinical data in COVID-19 patients. The correlation coefficients and the p-values for the correlation between ACE2, DKK1, and HMGB1 with the clinical and biochemical parameters in COVID-19 patients. ACE2 shows a significant correlation with SpO_2 ($\rho = 0.336$, $p<0.01$) and a negative correlation with albumin ($\rho = -0.197$, $p<0.05$). DKK1 have a significant correlation with HMGB1 ($\rho = 0.316$, $p<0.01$) and a negative correlation with albumin. Other parameters showed no significant correlation with the measured biomarkers.

Table 1. Demographic and clinical data of healthy controls (HC) and COVID-19 patients

Variables	HC (n = 30)	Patients (n = 60)	F/χ^2	p
Age years	39.57±9.68	42.75±10.10	2,358	0.061
Sex male/female	20/10	39/21	0.025	0.875*
BMI kg/m^2	27.07±4.83	28.83±5.81	2,094	0.156
Married/Single	23/7	47/13	2,147	0.088*
Body temperature °C	37.02±0.24	37.97±0.86	19,766	<0.001
Duration of disease/days	-	10.08±5.64	-	-
SBP mm Hg	120.23±2.62	129.07±15.12	12,432	0.002
DBP mm Hg	80.07±2.07	80.55±9.00	0.084	0.773
SpO_2	98.21±0.81	89.49±9.34	25,778	<0.001
LACTS %	-	40.67±11.84	-	-
O_2 therapy	0/30	30/30	22.5	<0.001
C-Reactive protein +/-	0/30	60/0	90,000	<0.001*
IgG +/-	0/30	44/16		<0.001
IgM +/-	0/30	39/21		<0.001
RT-PCR result +/-	0/30	48/12	51,429	<0.001
Urea mg/dl	30.73±7.26	48.83±11.68	31,178	<0.001
Creatinine mg/dl	0.68±0.17	0.91±0.33	372,699	<0.001
D-Dimer mg/l	3.49±2.12	13.49±2.76	131,932	<0.001
Glucose mg/dl	93.53±19.92	146.46±47.17	31,830	<0.001
Albumin g/l	44.57±4.70	31.23±5.49	169,259	<0.001
ACE2 ng/ml	2.46(0.73-3.14)	3.02(2.15-3.78)	11,938	0.013
DKK1 ng/ml	510.68 (399.80-629.53)	617.84(519.46-798.72)	12,919	<0.001
HMGB1 ng/ml	2.48 (2.18-3.35)	5.75 (5.09-6.87)	23,181	<0.001

Results expressed as mean±standard deviation for normally distributed data and binomial data were expressed as ratios. (*): analysis by Chi-squared test, ACE2: angiotensin-converting enzyme 2, BMI: body mass index, DBP: diastolic blood pressure, DKK1: Dickkopf-related protein-1, HMGB1: High-mobility group box 1 protein, IgG(M): immunoglobulin antibodies G (M), LACTS: lung abnormalities in CT-scan, SPO_2: blood oxygen saturation and, SBP: systolic blood pressure

Table 2. Biomarkers in COVID-19 patients divided into those with lung abnormalities in the CT-scan (+LACTS) and without lung abnormalities (-LACTS)

Parameters	Lung abnormalities in CT-scan		F	p-value
	Negative (-LACTS)	Positive (+LACTS)		
Age years	50.97±14.44	56.35±15.22	3,947	3,947
BMI kg/m^2	28.91±6.26	28.75±5.35	0.023	0.023
Temperature °C	37.91±0.82	37.96±0.89	0.081	0.081
SpO_2 %	91.55±5.40	87.58±11.57	5,675	5,675
Urea mg/dl	46.98±8.60	65.57±30.55	15.78	15.78
Creatinine mg/dl	0.84±0.17	1.18±0.82	7,493	7,493
D-dimer mg/l	2.12±3.83	2.11±3.18	<0.001	<0.001
Glucose mg/dl	183.87±75.28	192.59±83.24	4,713	4,713
Albumin g/l	28.00±6.94	26.52±5.94	1,589	1,589
ACE2 ng/ml	3.56±3.02	3.20±1.28	0.733	0.733
DKK1 ng/ml	811.44±494.01	722.73±316.19	1,391	1,391
HMGB1ng/ml	5.74±1.59	8.06±4.34	14,794	14,794

ACE2: angiotensin-converting enzyme 2, BMI: body mass index, DKK1: Dickkopf-related protein-1, HMGB1: High-mobility group box 1 protein, LACTS: lung abnormalities in CT-scan, and SPO_2: blood oxygen saturation

Table 3. Correlation between ACE2, DKK1, and HMGB1 with demographic and clinical data in COVID-19 patients

	ACE2	DKK1	HMGB1
Age	-0.085	-0.01	0.053
BMI	-0.021	-0.107	0.081
Sex	-0.088	-0.128	-0.049
Duration of disease	0.164	0.077	0.113
Temperature	0.133	-0.176	-0.134
SpO2	0.336**	0.055	0.014
O2 Therapy	-0.142	0.11	0.16
LACTS %	0.074	-0.043	0.131
D.dimer	0.093	-0.09	-0.06
Glucose	-0.086	0.061	-0.018
Albumin	-0.197*	-0.229*	-0.018
Urea	-0.15	-0.051	-0.003
Creatinine	0.012	0.119	-0.209
DKK1	0.053	1	0.316**
HMGB1	0.168	0.316**	1

*: Correlation is significant ($p<0.05$)
**: Correlation is significant ($p<0.01$)
ACE2: angiotensin-converting enzyme 2, BMI: body mass index, DKK1: Dickkopf-related protein-1, HMGB1: High-mobility group box 1 protein, LACTS: lung abnormalities in CT-scan, and SPO_2: blood oxygen saturation

4. Discussion

The most important findings of the present study are the significant increase in the serum biochemical parameters (glucose, urea, creatinine, D-dimer, DKK1, and HMGB1) in patients compared with the control group, as seen in Table 1. Also, COVID-19 patients were having a severe state of hypoxia revealed from the low values of SpO_2. In reality, most of the patients included in this study (82.7%) had hypoxemia, as measured by SpO_2 <92%. Furthermore, hypoxia can cause inflammation (Eltzschig *et al.* 2014) and increase ACE2 gene expression and protein levels in lung and renal tissues, all of which can contribute to the severity of COVID-19 (Shenoy *et al.* 2020). Peripheral oxygen saturation (SpO_2) is frequently reduced in COVID-19, particularly in more severe instances and stages of LACT (Luks and Swenson 2020).

ACE2 is expressed in lung alveolar epithelial cells, small intestinal enterocytes, arterial and venous endothelial cells, and arterial smooth muscle cells (Hamming *et al.* 2004). According to the results of the study, ACE2 is not only a receptor for SARS-CoV-2, but it is also engaged in post-infection control, which includes immune response and adaptive immune responses, and cytokine production, and viral genome replication (Fischer *et al.* 2017). Additionally, it is hypothesized that higher soluble ACE2 function as a competitive interceptor of SARS-CoV-2, delaying viral entrance into cells and protecting against lung damage (Batlle *et al.* 2020). SARS CoV2 induces ACE2 expression in various organs, including extensive beds of artery endothelial cells and even on the surfaces of many tissues other than the lung (Guan *et al.* 2020).

DKK1 was shown to be substantially expressed in COVID-19 patients compared to controls in one study, particularly in severe cases, and it is expressed by epithelial cells (Le *et al.* 2021). DKK-1 changes might be used as indicators of inflammation in other illnesses. This is the most likely explanation for the change in DKK1 levels in COVID-19 patients. With improved glycemic control and low-dose aspirin treatment, plasma DKK-1 levels are reduced (Lattanzio *et al.* 2014). ARDS is characterized by acute inflammation and increased coagulation cascade activity, increasing the risk of venous thromboembolic events (Paar *et al.* 2021). The DKK1 receptor, which inhibits canonical Wnt signalling, also serves as an entrance receptor for many viruses (Staring *et al.* 2018).

The level of HMGB1 was increased in COVID-19 patients compared to healthy controls, as seen previously (Fan *et al.* 2021). In one research, HMGB1 inhibition protected epithelial cells by stopping cytokine progress and also reduced viral replication (Gowda *et al.* 2021). In many human diseases, blood levels HMGB1 are increasing, and HMGB1 is proposed as a good marker for severity of disease and prognosis (Ishida *et al.* 2011). Chronic inflammation may have consequences on Treg and Th17 cells due to HMGB1 and RAGE signal (Subramanian *et al.* 2017). Increased the levels of HMGB1 are secondary to necrosis/cell destruction in adipose tissue when the chronic inflammatory stage is high (Subramanian *et al.* 2017). Pathogenesis and specific symptoms of the illness can play a significant role in HMGB1/RAGE signalling and pro-inflammatory cytokines (Lui *et al.* 2016). Inflammatory neurons, microcytes and astrocytes are indicated for HMGB1 to contribute to COVID-19 characteristics like fever, little taste loss and appetite through a Toll-like receptor 4 (TLR4) (Andersson *et al.* 2020).

The second finding is the presence of LACTS in more than half of COVID-19 patients and showed that about 40% of lungs were having in the CT-scan indicating the lung injuries due to the SAR-CoV-2 infection. These abnormalities involved GGO, crazy-paving, consolidation in addition to other minor abnormalities as illustrated elsewhere (Adams *et al.* 2020). Some biomarkers levels have been changed in patients with positive lung abnormalities (+LACTS) than the patients without lung injuries (-LACTS). SARS-CoV-2 is more common than previously believed to cause lung involvement (Palmowski *et al.* 2020).

A report showed that SARS-CoV-2 might use ACE2 tissue-protective mediator species-specific interferon-driven overexpression after lung damage to increase

infection (Ziegler *et al.* 2020). Lung damage leads to hypoxia, which may induce inflammation ACE2, and protein levels may be upregulated and may contribute to the degree of severity of COVID-19 in lung and renal tissues (Shenoy *et al.* 2020). Experimental investigations have shown that the medial use of HMGB1 by leucocytes in the lung is crucial in the mediation of acute lung damage (Huebener *et al.* 2019). Interestingly, HMGB1 controls autophagy and may be an acute pulmonary damage biomarker (Qu *et al.* 2019). Autophagy is one of the mechanisms that involve viral entrance and cell replication, and thus a viable new treatment strategy for COVID-19 has been proposed for this process (Yang and Shen 2020). In addition, expression of HMGB1 is augmented in illnesses linked to thrombosis, and was investigated with alveolar epithelial cells (Pittet *et al.* 2013). Severe inflammation has dual effects, and ACE2 is raised since the lung abnormalities grow, leading to decreased oxygen supplies and the SpO_2 ratio decreased (Al-Hakeim *et al.* 2021). The serum level of HMGB1 was correlated with both the severity of pathogen-associated tissue damage and excessive cytokine storm (Chan *et al.* 2012). In patients with COVID-19, the overproduced serum HMGB1 was related to unique cytokine storm characteristics(Entezari *et al.* 2014). Furthermore, HMGB1 serum was positive with a high CT score and oxygen demand at admission, which indicate the severity of acute lung damage and ARDS (Chen *et al.* 2020).

The regular measurement of urea and creatinine is crucial in COVID-19 for acute renal injury (AKI). In the COVID-19 patients with independent risk factors for in-hospital death after the age, sex, severity of illness, comorbidities and the leukocyte count, elevated baseline blood creatinine and urea were raised (Cheng *et al.* 2020). The results indicated that the incidence of renal illness during inpatient hospitalization and in AKI development in patients with COVID-19 is significant and linked to inpatient death. The AKI treatment aim in COVID-19 is strongly justified (Cheng *et al.* 2020). A substantial percentage of the patients in hospital admission exhibited subclinical indications of renal dysfunctions that were not yet AKI, e.g. 59% proteinuria, 44% hematuria, 14% higher blood urea nitrogen and 10% higher serum creatinine, albeit slightly but poorer than those with other infections (Li *et al.* 2020).

The other important findings of the present study are the significant correlation between ACE2 with SpO_2 and the negative association with albumin. DKK1 have a significant correlation with HMGB1and a negative correlation with albumin. These correlations revealed the dependence of the level of these biomarkers with the hypoxia state and the increase of immunoglobulins fraction of plasma proteins that causes a reduction in the plasma albumin. Albumin and prealbumin level abnormalities are frequent in COVID-19 patients and may predict poor prognosis and should be carefully evaluated in clinical practice (Li *et al.* 2020). Hypoalbuminemia is prevalent in COVID-19 individuals and can be seen at an early stage of the illness. It is only linked to inflammatory indicators and clinical outcome (Bassoli *et al.* 2021). There has been minimal research on the relationship between blood albumin levels and COVID-19 disease severity (Kheir *et al.* 2021). In COVID-19 patients, hypoalbuminaemia may serve as a severity measure of epithelial-endothelial damage (Wu *et al.* 2021). Other results revealed that fibrinogen and D-dimer levels were more significant in the severe patients, whereas platelets count and albumin levels were significantly lower (Bi *et al.* 2020).

In conclusion, COVID-19 patients are having an increase in the serum biochemical parameters (glucose, urea, creatinine, D-dimer, ACE2, DKK1, and HMGB1), in addition to a reduction in SpO_2 and albumin. COVID-19 patients had increased ACE, DKK1, and HMGB1, indicating that these biomarkers' pathways are involved in disease development. A large fraction of COVID-19 patients has had a sizeable LACTS percentage who were older and had lower SpO_2 than those without LACTS. LACTS patients had significantly higher blood levels of urea, creatinine, glucose, and HMGB1. ACE2 has a significant relationship with SpO_2 but a negative relationship with albumin. DKK1 has a significant relationship with HMGB1 and a negative relationship with albumin. The presence of LACTS and hypoxia in COVID-19 patients is linked to renal damage (as shown by elevated urea and creatinine levels) and serum HMGB1.

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