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Changes in liver enzymes and association with prognosis in patients with COVID-19: a retrospective case–control study

Farhad Shaveisi-Zadeh[1], Bahram Nikkho[2], Mohamaad Bagher Khadem Erfan[3], Amir Amiri[1], Ali Azizi[4], Nasrin Mansouri[1], Mitra Tarlan[1] and Zahra Rostami-Far[5]

Abstract

Objective: COVID-19 has recently emerged as a serious threat to global health. This study examined the laboratory investigations of patients with COVID-19, with an emphasis on liver enzymes.

Methods: This retrospective, single-center study was performed on patients with COVID-19 who were admitted to Imam Reza Hospital, Iran from March 2020 to February 2021. Laboratory tests included a complete blood cell count, white blood cell (WBC) count, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), lymphocyte/monocyte ratio, and levels of aspartate aminotransferase, alanine aminotransferase (ALT), and alkaline phosphatase. Patient survival was among the outcome measures investigated in association with laboratory findings.

Results: We enrolled 77 patients with COVID-19 and 63 healthy controls. In comparison with the control group, patients with COVID-19 showed COVID-19 increased ALT, WBC, neutrophils, NLR, and PLR, and decreased platelet counts and lymphocytes.

[1]Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Department of Pathology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
[3]Department of Parasitology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
[4]Department of Community Medicine, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
[5]Cellular and Molecular Research Center, Research Institute for Health Development, Kurdistan University of medical science, Sanandaj, Iran

Corresponding author:
Zahra Rostami-Far, Cellular and Molecular Research Center, Research Institute for Health Development, Kurdistan University of Medical Science, Sanandaj 6714415153, Iran.
Email: z.rostamifar@gmail.com

Conclusion: Although elevated levels of AST, NLR, PLR, and LMR were found in patients with COVID-19, they were not linked to mortality. Given the presence of AST in other tissues, the influence of SARS-CoV-2 on the liver should be interpreted with caution.

Keywords
SARS-CoV-2, COVID-19, liver enzyme, liver disease, liver injury, case–control study

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Introduction

The novel coronavirus (COVID)-19 disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first detected in Wuhan, China, spread rapidly throughout the world and was labeled a pandemic by the World Health Organization on 11 March 2020.

The clinical manifestations of COVID-19 in symptomatic patients typically include fever, fatigue, cough, and other signs of respiratory tract infections.[1,2] Severely affected patients show symptoms of pneumonia with unusual findings on chest computed tomography (CT), related to complications such as severe acute respiratory distress syndrome, acute heart injury, organ failure, and death.[3] Several studies have investigated the association between COVID-19 infection and blood biomarkers including leukopenia,[4] elevated high-sensitivity C-reactive protein (CRP) levels, elevated erythrocyte sedimentation rates (ESRs),[5] and abnormal liver enzymes.[4,5]

Similar to other coronavirus-related SARS, liver damage is a growing problem of COVID-19. Previous studies found that up to 60% of patients experienced liver injury, with viral nucleic acid and damage detected in liver biopsies.[6–8] Higher levels of alanine aminotransferase (ALT), fewer platelet counts, and lower albumin levels were also associated with a greater risk of death in patients with COVID-19.[9] In most of the severe cases, blood concentrations of pro-inflammatory cytokines such as tumor necrosis factor, interleukin (IL)-6, IL-1, and CRP were elevated, suggesting that cytokine storm syndrome may be linked to disease severity.[10,11] Moreover, the SARS-CoV-2 virus was recently shown to bind to angiotensin-converting enzyme 2 (ACE2) on cholangiocytes, causing cholangiocyte impairment and inducing a systemic inflammatory response that results in liver injury.[12]

It remains unclear how crucial liver damage is in the novel SARS-CoV-2 outbreak. Therefore, this study evaluated the clinical importance of liver function changes and their association with survival in patients with COVID-19.

Patients and methods

This retrospective, single-center study enrolled patients with COVID-19 who were admitted to Imam Reza Hospital, Kermanshah, Iran from March 2020 to February 2021. Ethical approval for this study was obtained from the ethics committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1400.065). The need for informed patient consent was waived by obtaining authorization from the institutional review board committee. The reporting of this study conforms to STROBE guidelines.[13]

We reviewed the medical records of 120 patients with COVID-19 selected by random sampling. Of these, 43 were excluded because they also had infectious liver disease (n = 5), cirrhosis (n = 7), hepatocellular carcinoma (n = 1), fatty liver (n = 20), chronic hepatitis B (n = 2), or had a history of alcohol abuse (n = 8). A simple random sampling method was used to select 63 healthy controls from individuals referred to the hospital for routine check-ups. Controls and cases were sex- and age-matched, and had the same sampling times.

COVID-19 assessment

Infectious disease and internal medicine clinicians made the diagnosis of COVID-19 based on clinical signs, a chest computed tomography scan, and a positive result from SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) using a real-time PCR assay (Kogene Biotech, Seoul, Korea) with RNA samples from the nasopharynx and throat.

Patients with respiratory rates >30 breaths/minute, SpO_2 < 93% on room air, or PaO_2/FiO_2 ≤300 mm Hg were categorized as severe cases (with acute lung injury), while other patients were classified as moderate cases.

Subject evaluation

Clinical and epidemiologic data were collected from patient medical histories. Initial laboratory assessments included a complete blood count, an assessment of coagulation function, and routine biochemical and liver function tests including alkaline phosphatase (ALP), aspartate aminotransferase (AST), ALT, and lactate dehydrogenase. No further sampling was carried out. The laboratory profile was monitored regularly based on the clinical progress of each patient. Reference values for AST, ALT, ALP, NLR, PLR, and LMR were based on previous reports.[14]

With respect to the RT-PCR data, the recorded cycle threshold associated with FAM (RdRP gene) and Hex (N gene) channels were collected to investigate the association with other parameters. Mortality was used as a prognostic factor.

RT-PCR assay

Quantitative RT-PCR on stored RNA samples to amplify SARS-CoV-2 RdRp, E, and N genes was carried out using the PowerChek™ 2019-nCoV real-time PCR kit (Kogene Biotech Co. Ltd., Seoul, Republic of Korea) according to the manufacturer's instructions. Target viral gene amplification was carried out using the following conditions: cDNA synthesis at 50°C for 30 minutes, predenaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s.

Sample size calculation

The sample size was calculated by substituting values of AST (mean, standard deviation [SD]) for patients with COVID-19 and controls obtained by Omrani–Nava[15] into a simplified formula to obtain a difference in means of power of 80% and a confidence interval (CI) of 95%. The sample size for each group was thus estimated to be 26:

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \left(S_1^2 + S_2^2\right)}{\left(X_1 - X_2\right)^2} \simeq 26 \quad (1)$$

Statistical analysis

All analyses were performed using SPSS software version 20 (IBM Corp., Armonk, NY, USA). Continuous data were expressed as means ± SD. Normal distribution was evaluated by the Kolmogorov–Smirnov

test. Differences between continuous variables were determined using the Student's t-test. The Mann–Whitney U test was used when variances were not normally distributed. A chi-squared test (χ^2 test) was performed on qualitative variables. The relationship between mortality and qualitative variables was examined by logistic regression analysis. Parameter sensitivity and specificity were determined using the receiver operating characteristic curve. The relationship between the reported cycle threshold in FEM and Hex RT-PCR channels and hepatic enzymes was determined by the Pearson coefficient of correlation. Significance was defined as $P < 0.05$.

Results

The study was performed on 77 patients with COVID-19 and 63 healthy controls. The mean age of the patients was 61.58 ± 17.76 years. There were 35 (45%) female patients and 42 (54%) male patients in the COVID-19 group, and 22 (28%) did not survive.

In comparison with control groups, patients with COVID-19 had significantly increased ALT levels ($P < 0.001$), although there was no significant difference in the levels of AST or ALP. Elevated levels of ALT were reported in 37 patients (49%), elevated levels of AST in 31 (41%), and elevated levels of ALP in 10 (13%). Laboratory parameter comparisons demonstrated significantly higher white blood cell counts (WBCs), neutrophil counts, neutrophil/lymphocyte ratios (NLR), and platelet/lymphocyte ratios in patients compared with controls, and significantly lower lymphocyte/monocyte ratios (LMRs), and lymphocyte counts ($P < 0.05$; Table 1).

Among patients with COVID-19, 57 (74%) were classified as having severe disease at admission. The mean age of severe cases was 61.85 ± 17.40 years, and 27 (47%) were male. No significant difference was observed in biochemical and laboratory variables between patients with moderate and severe COVID-19 (Table 2). Similarly, logistic regression analysis found no association between biochemical parameters and COVID-19 mortality (Table 3). Additionally, the Pearson correlation coefficient showed there was no significant relationship between cycle threshold reported in RT-PCR FEM and Hex channels and liver enzymes (Pearson correlation = 0.000).

Discussion

Coronaviruses are a group of viruses that cause respiratory and gastrointestinal diseases in both animals and humans.[16] They mainly affect the upper respiratory tract, causing moderate to severe infections ranging from the common cold to pneumonia.[17] Middle East respiratory syndrome-related coronavirus and SARS-CoV were previously linked to respiratory tract infection outbreaks in 2012 and 2002, respectively.[18,19] Although SARS-CoV-2 has a lower mortality rate than other viral family members, its high contagiousness and tendency to survive on surfaces for hours to days prompted global health policymakers to pay special attention to COVID-19.[20]

In the present study, we demonstrated elevated levels of AST, NLR, PLR, and LMR in patients with COVID-19; however, they were not correlated with mortality.

Both SARS-CoV-2 and SARS-CoV have been reported to use the ACE2 receptor to enter the target host cell, and then to ultimately infect the upper respiratory tract and lung cells.[2] *In vitro* experiments have shown that SARS-CoV causes direct liver damage,[21] and liver damage in patients infected with SARS-CoV-2 is thought to be caused by the virus itself.[22]

Lactate dehydrogenase (35.1%), AST (21.6%), ALT (18.2%), gamma-glutamyl transferase (17.6%), overall bilirubin (6.1%), and ALP (18.2%) were the most

Table 1. Clinical characteristics and laboratory results of study participants.

Variable	Covid-19 group n (%)	Control group n (%)	Reference value	P-value
Age (years, mean ± SD)	61.58 ± 17.76	53.07 ± 11.43		0.002
≥60	35 (45.5%)	10 (15.9%)		<0.001
<60	42 (54.5%)	53 (84.1%)		
Sex				0.246
Male	35 (45.5%)	26 (41.3%)		
Female	42 (54.5%)	37 (58.7%)		
AST (mean ± SD, IU/L)	139.45 ± 586.2 75	23.14 ± 7.9 63	5–40	<0.001
ALT (mean ± SD, IU/L)	77.62 ± 259.5 75	30.48 ± 17.53 62	5–40	0.142
ALP (mean ± SD, IU/L)	227 ± 180.8 72	200.26 ± 78.77 57	64–306	0.743
RBC (mean ± SD, million/mm^3)	5.24 ± 3.78 74	4.79 ± 0.6 7 63	Male: 4.5–6.3 Female: 4.2–5.4	0.702
WBC (mean ± SD, /mm^3)	9.39 ± 5.1 74	6.76 ± 2.18 63	4000–10000	<0.001
HCT (mean ± SD, %)	41.1 ± 4.3 74	40.44 ± 4.89 63	Male: 39–52 Female: 36–46	0.541
Platelets (mean ± SD, /mm^3)	215.82 ± 89.15 74	227.73 ± 58.60 63	140,000–440,000	0.131
Lymphocytes (mean ± SD, %)	1.18 ± 0.88 74	2.13 ± 0.78 63	20–40	<0.001
Neutrophils (mean ± SD, %)	7.78 ± 4.73 74	4.04 ± 1.98 63	40–60	<0.001
NLR (mean ± SD)	8.84 ± 6.5 74	2.69 ± 3.81 63	1.70 ± 0.07	<0.001
PLR (mean ± SD)	235.15 ± 137.02 74	139.02 ± 152.02 63	117.05 ± 47.73	<0.001
LMR (mean ± SD)	3.65 ± 4.1 74	4.53 ± 2.42 63	11.15 ± 3.14	0.03

SD: standard deviation; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; RBC: red blood cell; WBC: white blood cell; HCT: hematocrit; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte/ ratio; LMR: lymphocyte/monocyte ratio.

commonly elevated liver function tests in a previous analysis of 148 patients with COVID-19.[23] Moreover, Chen et al. observed various degrees of liver dysfunction in 99 patients with COVID-19, including elevated ALT and AST levels in 28% and 35% of patients, respectively, total bilirubin in 18%, and reduced albumin levels in 98%.[24] Abnormal levels of ALT and AST were reported in 30.6% and 61.6% of patients, 14.6% and 12.1% of patients, and 22.2% and 21.3% of patients, respectively, in different studies.[25–27] Here, we reported elevated levels of ALT, AST, and ALP in 49%, 41%, and 13% of patients, respectively.

AST elevation is more commonly seen than ALT elevation, indicating that AST may originate from places other than the liver.[28] It is also noteworthy that, while ALT is a specific marker for liver injury, AST changes are non-specific because of

Table 2. Clinical characteristics and laboratory results of patients with moderate and severe COVID-19.

Variable	Moderate COVID-19 group	Severe COVID-19 group	Reference value	P-value
Age (years, mean ± SD)	61.86 ± 17.4	60.8 ± 19.18		0.8
≥60	27	10		0.5
<60	30	10		
Sex (%)				0.3
Male	27	8		
Female	30	12		
AST (mean ± SD, IU/L)	141.98 ± 668.42	132.5 ± 261.8	5–40	0.9
	55	20		
ALT (mean ± SD, IU/L)	76.1 ± 289.1	82.16 ± 146.98	5–40	0.9
	56	19		
ALP (mean ± SD, IU/L)	220.2 ± 187.1	246 ± 165.34	64–306	0.5
	53	19		
LDH (mean ± SD, U/mL)	866.65 ± 493.93	1036.2 ± 770		0.5
	23	6		
RBC (mean ± SD, million/mm^3)	5.43 ± 4.37	4.72 ± 0.71	Male: 4.5–6.3 Female: 4.2–5.4	0.4
	55	19		
WBC (mean ± SD, /mm^3)	9.58 ± 5	8.85 ± 5.15	4000–10000	0.5
	55	19		
HCT (mean ± SD, %)	41.11 ± 4.28	40.88 ± 4.57	Male: 39–52 Female: 36–46	0.8
	55	19		
NLR (mean ± SD)	8.71 ± 6.73	9.25 ± 5.94	1.70 ± 0.07	0.7
	55	19		
PLR (mean ± SD)	250.33 ± 130.67	229.9 ± 139.96	117.05 ± 47.73	0.5
	55	19		
LMR (mean ± SD)	3.15 ± 1.98	5.11 ± 7.35	11.15 ± 3.14	0.07
	55	19		
CRP	3.02 ± 1.1	2.64 ± 1.39	1–4	0.3
	44	14		
Pt (mean ± SD, /mm^3)	12.79 ± 2.22	12.71 ± 0.83	12.5	0.7
	50	14		
PTT (s)	38.29 ± 28.52	37.33 ± 7.2	24–35	0.8
	49	14		
D-dimer	positive 2 (18.2%); negative 2 (25%)	positive 9 (81.8%); negative 6 (75%)		0.574
	15	4		
ESR	38.1 ± 15.92	36.25 ± 19.71	Male: <15 mm/h; Female: <20 mm/h	0.7
	30	8		

ALP: alkaline phosphatase; LDH: lactate dehydrogenase; RBC: red blood cell; WBC: white blood cell; HCT: hematocrit; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte/ratio; LMR: lymphocyte/monocyte ratio; CRP: C-reactive protein; Pt: platelet; PTT: partial thromboplastin time; ESR: erythrocyte sedimentation rate.

Table 3. Association between blood biochemistry and COVID-19-related mortality.

Variable	Odds ratio	95% CI		P-value
		Lower	Upper	
AST (elevated) 3	1.26	0.1	20.7	0.9
ALT (elevated)	0.21	0.1	29.73	0.5
ALP (elevated)	22.17	0.11	324.5	0.3
CRP (+1 Positive)	0.41	0.1	38.56	0.5
CRP (+2 Positive)	0.19	0.1	39.19	0.7
CRP (+3 Positive)	0.2	0.1	4.01	0.5
ESR (elevated)	0.9	0.1	1.2	0.1
NLR (elevated)	0.3	0.2	20.1	0.9
PLR (elevated)	0.1	0.1	1.5	0.09
LMR (elevated)	0.7	0.5	3.2	0.5

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte/ratio; LMR: lymphocyte/monocyte ratio; CI: confidence interval.

their widespread expression[29,30] which includes the heart, skeletal muscle, erythrocytes, and the liver.[31]

We found no significant difference in liver enzymes or other parameters between patients affected with moderate and severe COVID-19. This is in line with findings reported by Wu et al.,[32] while Huang et al. showed that levels of liver ALT and total bilirubin were considerably higher in patients needing urgent and intensive care than in other patients with COVID-19.[33]

In the present study, we observed an elevated WBC, platelet count, and NLR in patients with COVID-19 compared with controls. Neutrophils are the first leukocyte to arrive at the site of viral infection, penetrate the infected cell, and mediate a host defense barrier against viral infection. Subsequent rises in serum levels of TNF, IL-8, IL-6, interferon-γ, and granulocyte colony-stimulating factor further stimulate neutrophils, causing them to proliferate and migrate to virally infected areas.[34,35] Platelet counts in patients with COVID-19 were previously shown to be considerably lower than in controls,[36] but these should be considered together with lymphocyte counts when interpreting changes in PLR.[37] A decrease was recorded in the peripheral blood lymphocyte count of critically ill patients with COVID-19. [37] A systemic inflammatory response suppresses T cell-mediated immunity, resulting in lower levels of T lymphocytes.[38] Eslamijouybari et al. [39] also reported significantly higher NLR and PLR in patients with COVID-19 compared with controls, but only NLR was associated with disease severity. Moreover, Qu et al.[40] reported a significant difference in PLR between critically ill patients with severe and non-severe COVID-19 symptoms, while Yang et al.[41] showed that NLR is a reliable indicator and prognostic marker, and that LMR was considerably lower in patients with COVID-19; however, its discriminatory power and area under the curve (AUC) were also low. Thus, this score is unhelpful in diagnosing COVID-19.

Our study was limited by being confined to a single center because Imam Reza Hospital is a community teaching hospital that is not part of a broader healthcare system. Additionally, the interpretation of our results is limited by the small sample size, so our findings should be validated in a larger multi-center study. Nevertheless, the inclusion of a control group, the presence of other diseases that affect liver enzyme tests, and the investigation of the association between liver enzyme tests and results are all strengths of this study.

Conclusion

Further work is needed to fully understand the hepatocellular involvement caused by COVID-19. Although this study found elevated levels of AST, NLR, PLR, and LMR in patients with COVID-19, they were not linked to mortality. Given the role of the immune system in this occurrence and the presence of AST in other organs, the effect

of SARS-Cov2 on the liver should be approached with caution.

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Declaration of conflicting interest

The authors have no competing interests to declare.

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ORCID iDs

Ali Azizi https://orcid.org/0000-0002-7128-4181
Nasrin Mansouri https://orcid.org/0000-0002-8716-0909
Zahra Rostami-Far https://orcid.org/0000-0001-9680-6671

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