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Analyzing Correlation of Clinical Severity of COVID-19 with Other Biochemical Parameters: A Retrospective Study from Pakistan

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The third wave of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing damage all over the world, especially in Pakistan and India. Although vaccines are available and preventive measures are being taken, but SARS-CoV-2 is unstoppable. Currently, there are around 841,636 positive cases in Pakistan and 18,429 deaths, whereas, in India, both are high. From April 8th to 12th, 2021, nasopharyngeal swabs of 190 patients were submitted to PRL (PACP) lab for the SARS-CoV-2 testing, and blood samples were collected at the Mayo Hospital lab for ferritin, D-dimers, lactate dehydrogenase (LDH), and C-reactive protein (CRP) testing. This study observed that coronavirus disease 2019 (COVID-19) was more likely in individuals aged 51-60 than 61-70. In addition, our study found that COVID-19 patients exhibited a statistically significant increase in levels of ferritin, D-dimers, LDH, and CRP. In addition, this study found that COVID-19 patients had significantly higher levels of ferritin, D-dimers, LDH, and CRP. Our study revealed that SARS-CoV-2 relapsed. Furthermore, we concluded that these biochemical parameters are useful indicators for severity of COVID-19.

Keywords: biochemical markers; clinical severity; COVID-19; Lahore; SARS-CoV-2
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Introduction

In December 2019, an outbreak of corona virus infection appeared in Wuhan, a central city in China. Due to the ease of transmission and prolonged persisting time, corona virus infection has spread globally. It was proposed that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the β-coronavirus genus, triggered the epidemic (Al-Qahtani 2020). SARS-CoV-2 was previously known as the 2019-novel virus (2019-nCoV) before it was given the title as SARS-CoV-2 (Zheng 2020).

When preparing this article, the third wave of SARS-CoV-2 is currently creating havoc in South Asia, mainly in India. There are around 841,636 positive cases in Pakistan with 18,429 deaths, whereas, in India, positive cases are around 21,070,852 with 230,151 deaths. Despite vaccination and precautions, SARS-CoV-2 is uncontrolled.

Symptoms of coronavirus disease 2019 (COVID-19) appear 3-7 days after viral transmission (Cleri et al. 2010). Although there are several symptoms associated with the infection of SARS-CoV-2, the most common symptoms are dry cough, fever, nausea, dizziness, muscle aches, sore throats, weakness, chills, chest tightness, and fatigue (Baj et al. 2020).

Studies have shown that exposure to SARS-CoV-2 affects several parameters. For example, COVID-19 patients had increased eosinophil count, C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), and white blood count (WBC) (Khourssaji et al. 2020).

Several studies used clinical biomarkers like partial thromboplastin time (PTT) and activated partial thromboplastin time (APTT) to predict COVID-19 severity. In addition, neutrophils and antibodies have been found to help combat SARS-CoV-2 in several studies. (Fouladseresht

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et al. 2021). Cardiovascular prognostic factors also play a role in determining the morbidity of COVID-19 (Aboughdir et al. 2020). In this study, we observed the relation between ferritin, D-dimers, LDH, and CRP with the severity of COVID-19.

Materials and Methods

Study design

This retrospective study was conducted at the Mayo Hospital Institute of Public Health, Lahore, Pakistan. This research analyzed 190 samples. These samples were collected from April 8th to 12th, 2021. People tested for SARS-CoV-2 in Mayo Hospital, Lahore in between April 8th to 12th, 2021, were screened for inclusion in the study. The enrolled subjects were Lahore residents. A convenient mode of sampling was attained. During data collection, informed consent was obtained from patients. Exclusion criteria included subjects without recorded ferritin, D-dimer, CRP, and LDH. A schematic diagram showing the study design has been presented (Fig. 1). The enrolled subject's symptoms and the positivity rate have been represented in Table 1.

Ethical approval

Informed consent was obtained from patients, and the institute's ethical committee approved the study.

Sample collection

Nasopharyngeal swab and blood samples of 190 patients were collected based on the similarity of their symptoms to viral pneumonia.

All patients' nasopharyngeal swabs were collected and put in Viral Transport Medium (VTM) for safe transfer to the lab for SARS-CoV-2 detection. In addition, patients' blood samples were collected in microtubes containing lithium heparin to evaluate biochemistry parameters.

Laboratory testing

Each nasopharyngeal swab sample was submitted to PRL (PACP) lab with a barcode number. Cobas system (FDA, CE-IVD) of Roche was used to perform real-time PCR to detect the SARS-CoV-2 virus. The Mayo Hospital pathology lab in the biochemistry department collected the blood samples. Each sample was centrifuged for 15 minutes at 2,500 r.p.m. After centrifugation, each sample was sent through an automated machine, Backmann Coulter AU680 chemistry analyzer, to evaluate four biochemistry parameters: ferritin, CRP, D-dimers, and LDH.

Data analysis

The data was all obtained in excel. Excel 2013 (Microsoft Office Professional Plus) was utilized. All mean values and standard deviations (SD) were computed.

Statistical analysis

Statistical analysis was done. The P-value for the data was determined using SPSS software. P-value < 0.05 was considered as statistically significant.

Results

Our study included 190 individuals of all ages and sexes. 129 of 190 subjects were tested positive for SARS-

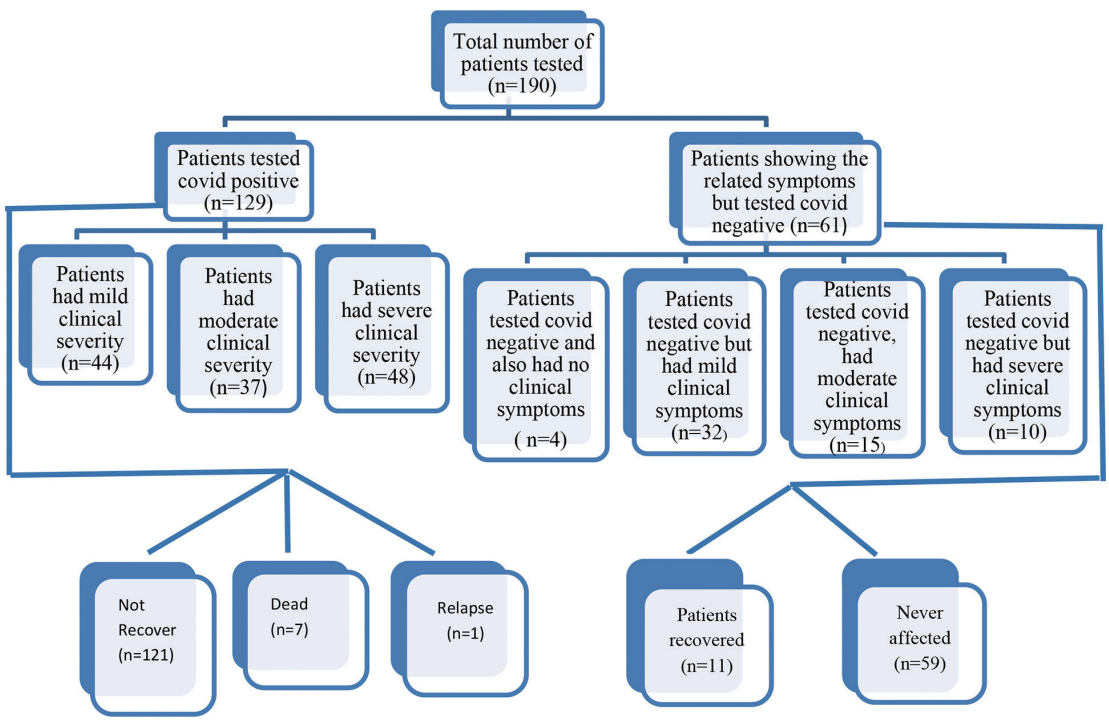

Fig. 1. Schematic representation of study design.

Table 1. Detail of the symptoms of COVID-19 in the enrolled subjects along with the positivity rate of PCR test for SARS-CoV-2.

Symptoms of patients	National guidelines	PCR Results for SARS-CoV-2					
		Negative (n)	Negative (%)	Positive (n)	Positive (%)	Total (n)	Total (%)
Asymptomatic	Asymptomatic	4	6.56%	0	0%	4	2.11%
Mild	Respiratory rate < 24/min, SpO_2 > 94% at room temperature	32	52.46%	44	34.11%	76	40%
Moderate	Respiratory rate 24-30 /min, SpO_2 90-94% at room temperature	15	24.59%	37	28.68%	52	27.37%
Severe	Respiratory rate > 30 /min, SpO_2 < 90% at room temperature	10	16.39%	48	37.21%	58	30.53%
Grand total		61	100%	129	100%	190	100%

CoV-2. Infected patients' blood showed a higher level of CRP (98%), LDH (95%), ferritin (79%), and D-dimers (98%).

71 males out of 103 were tested positive, whereas 58 females out of 87 were tested positive. Sex did not seem to matter much in terms of prevalence. The age group 51-60 years had the highest incidence of COVID-19 (73.33%), followed by the age group of 61-70 years (79.07%) (Table 2).

We studied the clinical severity of positive patients. The majority of positive patients (37.2%) had severe symptoms, followed by mild symptoms (34.1%) (Fig. 2).

We looked through the medical histories of patients to see if they had ever been infected with SARS-COV-2. We also followed up patients to see how many recovered and how many died. We discovered 11 subjects who had previously tested positive for COVID-19 but had recovered. Most of the recovered patients were aged 41-60 years (64%). However, one patient had SARS-COV-2 re-exposure. (Shown in Fig 2 as relapse) After recovering from COVID-19, the patient tested positive again. 7 out of 121 positives died. Most of the deceased patients were aged 61-80 years (Fig. 3).

We compared the mean values of ferritin, D-dimers, CRP, and LDH of negative and positive patients. Comparing the mean values of these biomarkers, we discovered that positive patients had higher mean values (Table 3). Thus, these biomarkers helped assess the clinical severity. Severe patients showed higher mean levels of ferritin, D-dimer, CRP, and LDH than mild and moderate patients.

COVID-19 positive patients had higher values of these biomarkers than negative patients. Graphs comparing mean values of COVID-19 negative and positive patients of each biomarker separately. The results for ferritin, CRP, and LDH were statistically significant. However, for D-dimer, the results were not statistically significant. (P = 0.9) (Figs. 4, 5, 6 and 7). The receiver operating characteristic (ROC)

Table 2. Age-wise and sex-wise distribution of the PCR test positive for SARS-CoV-2.

Age interval	Total tested	Males tested	Males positive	Females tested	Females positive	Total positive tested	P-value
Up to 30	13	9	5 (55.56%)	4	1 (25.0%)	6 (46.15%)	0.31
31-40	19	12	5 (41.67%)	7	3 (42.86%)	8 (42.10%)	0.96
41-50	37	22	16 (8.42%)	15	9 (60.0%)	25 (67.57%)	0.42
51-60	60	26	18 (72.73%)	34	26 (76.47%)	44 (73.33%)	0.53
61-70	43	24	19 (79.17%)	19	15 (78.95%)	34 (79.07%)	0.98
71-80	16	10	8 (80.0%)	6	2 (33.33%)	10 (62.5%)	0.06
> 80	2	0	0 (0%)	2	2 (100.0%)	2 (100.0%)	< 0.05
Total	190	103	71 (68.93%)	87	58 (66.67%)	129 (67.89%)	

Data are shown as n (%).

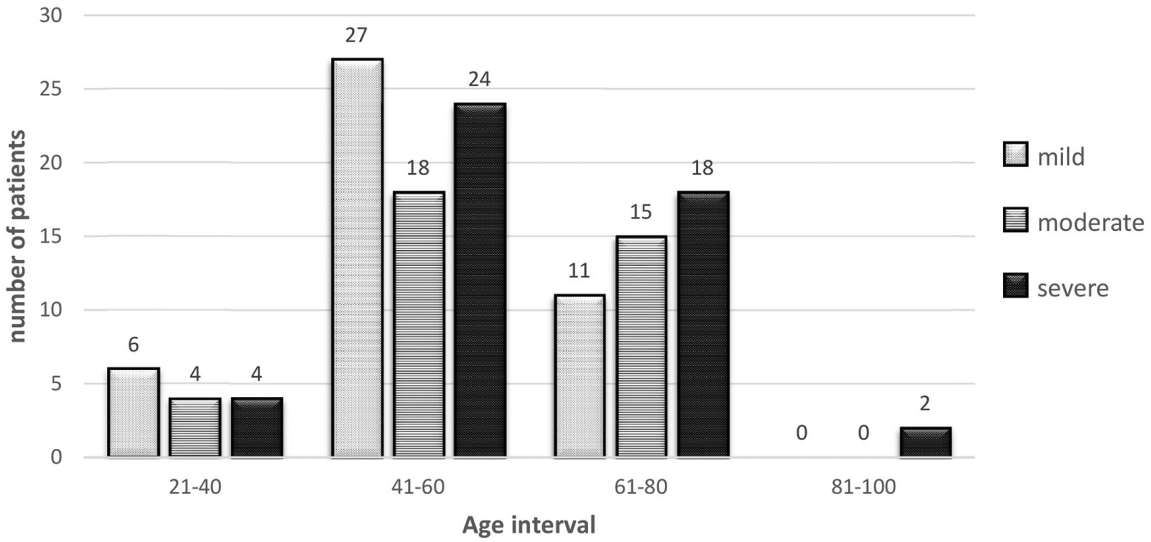

Fig. 2. Age-wise and condition-wise distribution of 129 positive patients for SARS-CoV-2.
Symptoms and age-wise distribution of infected people are represented. Most of the subjects enrolled in the study were belonging to the age group of 41-60 years; only two patients aged 81-100 years with severe conditions were observed. Most of the infected patients were showing severe symptoms.

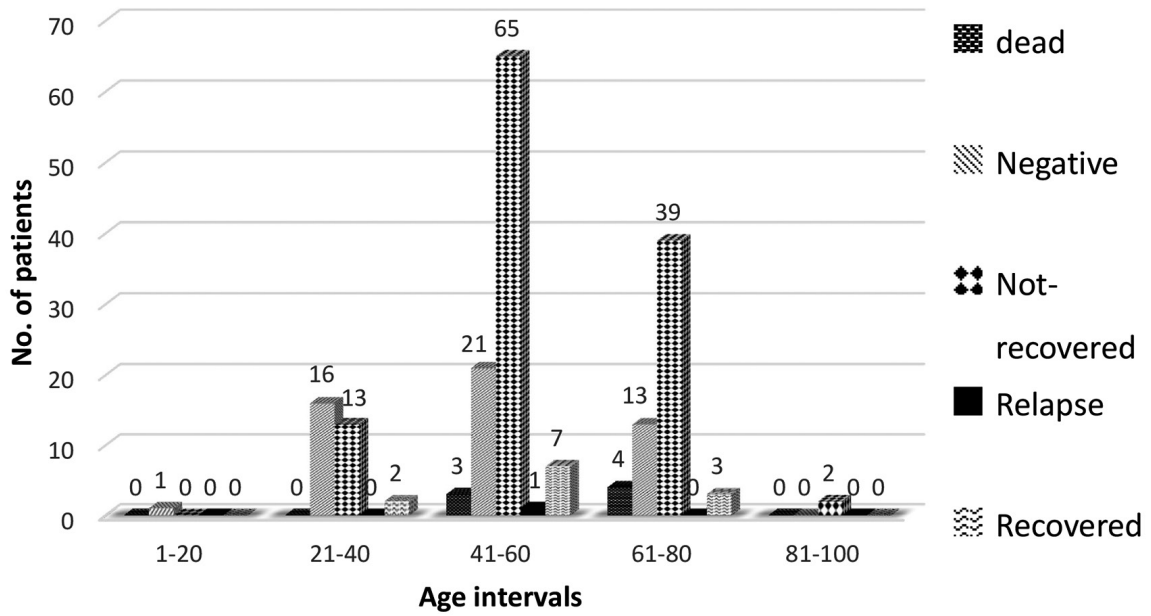

Fig. 3. Medical condition and age-wise distribution of 190 patients tested by PCR for SARS-CoV-2.
The medical conditions of the enrolled subjects have been represented. We found a low recovery rate as the data collection period was small. We observed one patient who tested positive for COVID-19 after clinical recovery and initial clearance of the virus. Negative subjects were enrolled as they were having close contact history with COVID-19 patients but were tested negative.

curves, along with the area under the curve (AUC) for ferritin, D-dimer, CRP, and LDH are represented (Fig. 8). The box plot of ferritin, D-dimer, CRP, and LDH in COVID-19 negative and positive patients are shown in Fig. 9.

We found that means values of ferritin, D-dimers, CRP, and LDH were higher in patients with severe symptoms than patients with mild and moderate symptoms. So we concluded that these biomarkers are directly linked to COVID-19 clinical severity. We compared the clinical severity of positive patients by sex to analyze and compare the results for more information (Table 4).

Discussion

During an infection, several parameters deviate from expected values. Therefore, determining the characteristics of such parameters during infection is critical for predicting infection severity and reducing mortality. This process of abnormal systematic response to an infection is termed sep-

Table 3. Mean values of biomarkers according to clinical severity of COVID-19 in PCR-positive and negative patients.

SR. no.	Clinical severity (n)	PCR results (n)	Ferritin (ng/ml)	D-dimers (μg/ml)	CRP (mg/L)	LDH (U/L)
1	Asymptomatic (4)	Negative (4)	170.75 ± 167.85	0.42 ± 0.30	0.53 ± 0.44	242.25 ± 156.75
		Positive (0)	0	0	0	0
2	Mild (76)	Negative (32)	176.9 ± 65.57	1.30 ± 0.63	20.81 ± 13.46	330.12 ± 66.26
		Positive (44)	732.73 ± 157.30	3.33 ± 1.30	73.42 ± 21.46	591.70 ± 90.04
		P value	0.0000007	0.006	0.0001	0.000001
3	Moderate (52)	Negative (15)	320.89 ± 156.19	2.66 ± 1.91	21.22 ± 19.31	383.8 ± 92.90
		Positive (37)	801.96 ± 205.82	3.69 ± 1.54	75.13 ± 23.11	657.70 ± 87.47
		P value	0.002	0.21	0.003	0.0002
4	Severe (58)	Negative (9)	449.64 ± 422.83	3.81 ± 3.47	44.42 ± 34.13	565.4 ± 128.95
		Positive (49)	819.85 ± 164.48	4.50 ± 1.32	77.6 ± 17.79	789.27 ± 96.59
		P value	0.03	0.33	0.05	0.02

CRP, C-reactive protein; LDH, lactate dehydrogenase.
Data are shown as mean ± SD.

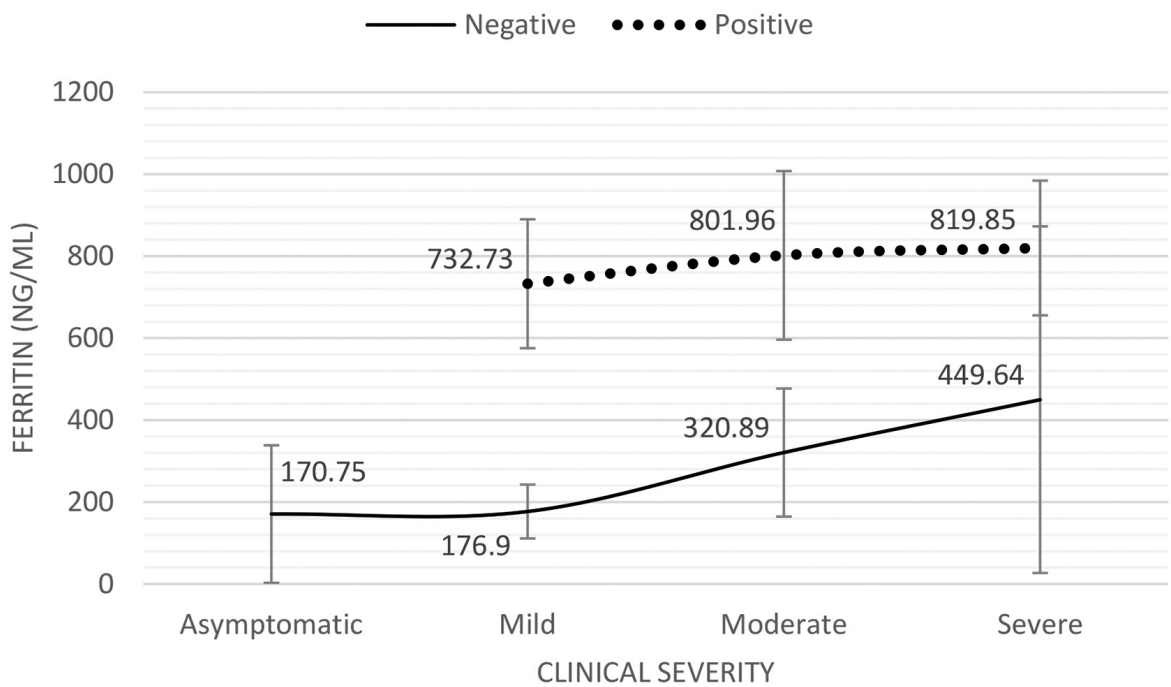

Fig. 4. Comparison of mean values of ferritin parameter between Negative and Positive patients of COVID-19.
The comparison between mean values of ferritin between COVID-19 negative and positive patients with respect to severity was found to be statistically significant ($P < 0.00001$).

sis. Several parameters are used to diagnose sepsis. The most well-known are lactate, CRP, and neutrophils (Faix 2013). Similarly, to access COVID-19 severity and minimize mortality, obtaining data related to infection is critical. Therefore, we decided to check if ferritin, CRP, LDH, and D-dimers were associated with the clinical severity of COVID-19.

Our research found the relation of D-dimers, CRP, LDH, and ferritin with the clinical severity of COVID-19. In addition, several studies on COVID-19 clinical biomarkers have been completed. For example, a study found a close relationship between COVID-19 severity and thrombin time, prothrombin time, fibrinogen, and activated partial thromboplastin time (Long et al. 2020).

The vaccination is ongoing, and the Pakistan government is doing its utmost to prevent the spread of SARS-

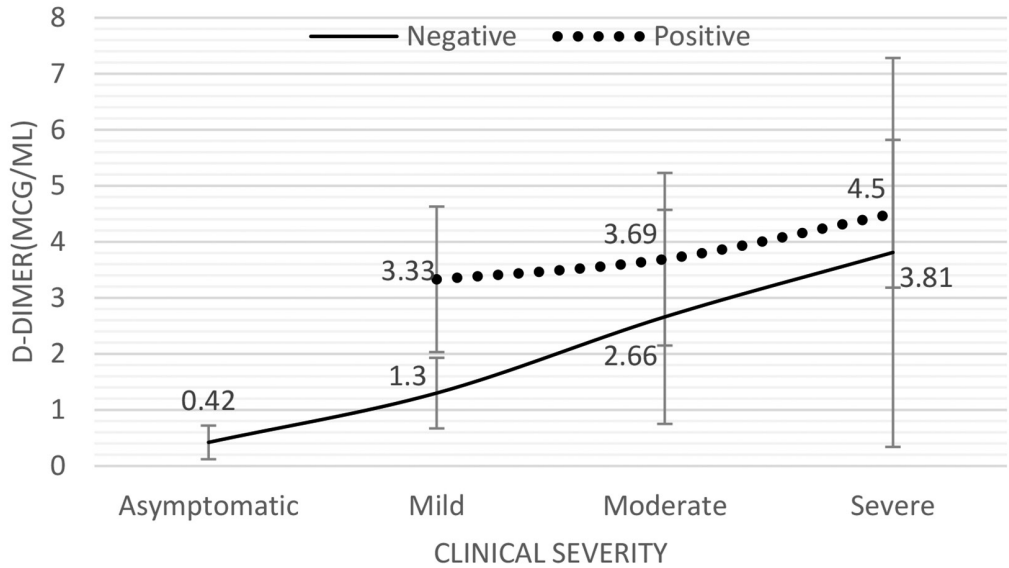

Fig. 5. Comparison of mean values of D-dimers parameter between Negative and Positive patients of COVID-19.
For D-dimer values the comparison between SARS-COV-2 negative and positive patients with respect to symptoms was not statistically significant (P = 0.9).

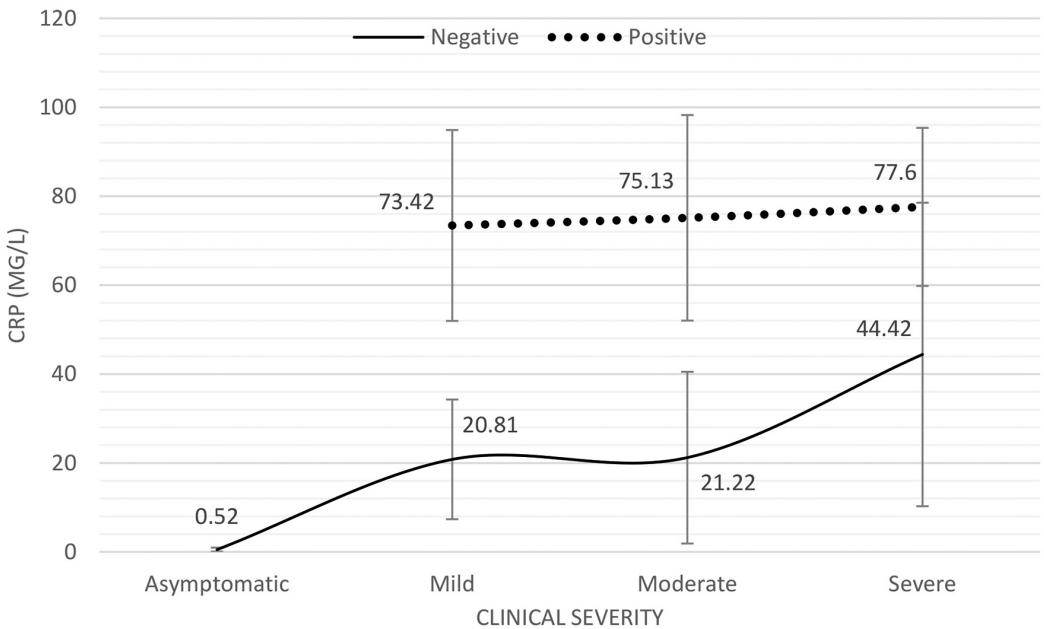

Fig. 6. Comparison of mean values of C-reactive protein (CRP) parameter between Negative and Positive patients of COVID-19.
For CRP values, the results were found to be statistically significant (P = 0.04).

CoV-2, but the positive rate remains high. Pakistan devised an innovative lockdown approach to minimize the economic damage. Previously, Pakistan halted trade with Iran and local flights to Iran, Iraq, and Saudi Arabia (Abid et al. 2020). Despite these efforts, SARS-COV-2 has not been completely contained. The unexpected spike in positivity rate might be due to a lack of knowledge in public or non-serious conduct toward SOPs. A survey-based study can uncover the real cause for SARS-COV-2 spread in Pakistan.

In this research, we discovered a significant incidence of COVID-19 in Lahore. Furthermore, we found a direct relationship between COVID-19 severity with ferritin, CRP, D-dimers, and LDH. Our study revealed that SARS-COV-2 relapsed. However, the recurrence rate among positive patients was modest (0.77%). More research is needed on the SARS-CoV-2 relapse pattern and the effects of co-morbidities on it. The mortality rate of patients who were re-infected after the initial clearance of SARS-CoV-2 can be studied. During the vaccination procedures, researchers can monitor the immune system response. Several survey-

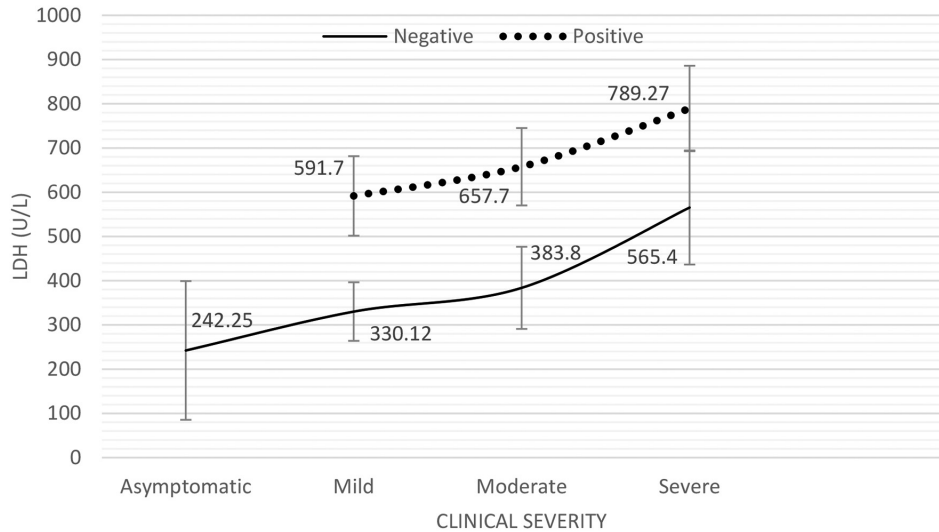

Fig. 7. Comparison of mean values of lactate dehydrogenase (LDH) parameter between Negative and Positive patients of COVID-19.
The comparison for the mean values of LDH was statistically significant as well (P < 0.0001).

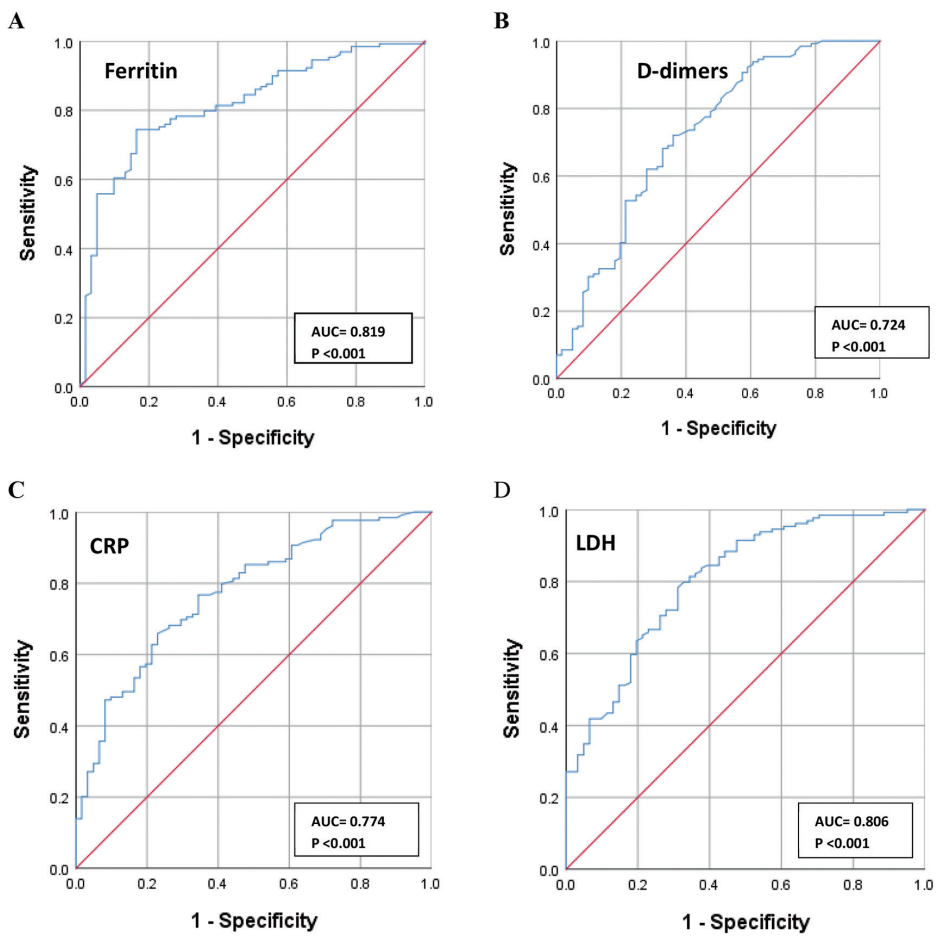

Fig. 8. Receiver operating characteristic (ROC) curve along with area under the curve (AUC) for ferritin, D-dimer, C-reactive protein (CRP), and lactate dehydrogenase (LDH) as a biochemical marker for COVID-19.
(A) The AUC for the ROC curve for ferritin values on admission against patient outcome was 0.819 [95% confidence interval (CI) 0.756-0.881, P < 0.001]. (B) The AUC for the ROC curve for D-dimer values on admission against patient outcome was 0.724 (95% CI 0.643-0.805, P < 0.001). (C) The AUC for the ROC curve for CRP values on admission against patient outcome was 0.774 (95% CI 0.705-0.843, P < 0.001). (D) The AUC for the ROC curve for LDH values on admission against patient outcome was 0.806 (95% CI 0.741-0.872, P < 0.001).

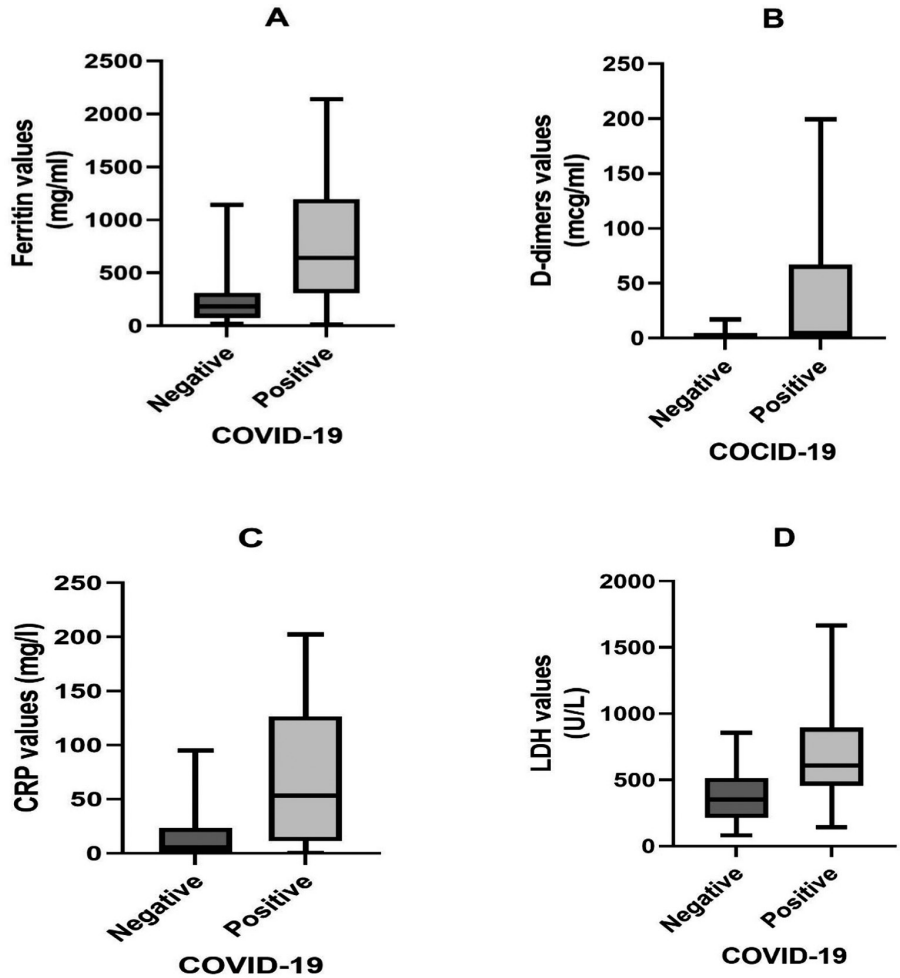

Fig. 9. Box plots of ferritin, D-dimer, C-reactive protein (CRP), and lactate dehydrogenase (LDH) in COVID-19 showing a significant difference between COVID-19 negative and positive patients.
(A) Ferritin; (B) D-dimer; (C) CRP; (D) LDH.

Table 4. Mean values of biomarkers in PCR-positive patients grouped by clinical severity of COVID-19 and sex.

SR. no.	Clinical severity of COVID-19	Sex	Ferritin (mg/ml)	D-dimers (μ/ml)	CRP (mg/L)	LDH (U/L)
1	Mild	Female	720.79 ± 221.23	4.67 ± 2.26	91.71 ± 31.62	648.62 ± 108.34
		Male	747.06 ± 244.62	1.71 ± 0.56	51.46 ± 27.94	523.4 ± 155.10
		P value	0.43	0.009	0.02	0.08
2	Moderate	Female	467.39 ± 245.24	4.34 ± 3.11	77.43 ± 42.43	602.8 ± 131.58
		Male	1,030.07 ± 276.18	3.255 ± 1.70	73.57 ± 29.14	695.14 ± 122.80
		P value	0.002	0.245	0.435	0.15
3	Severe	Female	652.88 ± 234.28	4.12 ± 1.98	93.18 ± 32.62	694.21 ± 151.68
		Male	929.24 ± 226.77	4.749 ± 1.85	67.39 ± 21.12	851.55 ± 127.26
		P value	0.04	0.32	0.07	0.06

CRP, C-reactive protein; LDH, lactate dehydrogenase.
Data are shown as mean ± SD.

based studies may also be done to learn more about people's behavior toward SARS-CoV-2 and the vaccination process in Pakistan.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

References

Abid, K., Bari, Y.A., Younas, M., Tahir Javaid, S. & Imran, A. (2020) Progress of COVID-19 epidemic in Pakistan. *Asia Pac. J. Public Health*, **32**, 154-156.

Aboughdir, M., Kirwin, T., Abdul Khader, A. & Wang, B. (2020) Prognostic value of cardiovascular biomarkers in COVID-19: a review. *Viruses*, **12**, 527.

Al-Qahtani, A.A. (2020) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): emergence, history, basic and clinical aspects. *Saudi J. Biol. Sci*., **27**, 2531-2538.

Baj, J., Karakula-Juchnowicz, H., Teresinski, G., Buszewicz, G., Ciesielka, M., Sitarz, E., Forma, A., Karakula, K., Flieger, W., Portincasa, P. & Maciejewski, R. (2020) COVID-19: specific and non-specific clinical manifestations and symptoms: the current state of knowledge. *J. Clin. Med*., **9**, 1753.

Cleri, D.J., Ricketti, A.J. & Vernaleo, J.R. (2010) Severe acute respiratory syndrome (SARS). *Infect. Dis. Clin. North Am*., **24**, 175-202.

Faix, J.D. (2013) Biomarkers of sepsis. *Crit. Rev. Clin. Lab. Sci*., **50**, 23-36.

Fouladseresht, H., Doroudchi, M., Rokhtabnak, N., Abdolrahimzadehfard, H., Roudgari, A., Sabetian, G. & Paydar, S. (2021) Predictive monitoring and therapeutic immune biomarkers in the management of clinical complications of COVID-19. *Cytokine Growth Factor Rev*., **58**, 32-48.

Khourssaji, M., Chapelle, V., Evenepoel, A., Belkhir, L., Yombi, J.C., van Dievoet, M.A., Saussoy, P., Coche, E., Fillee, C., Constantinescu, S.N., Rodriguez-Villalobos, H., Defour, J.P. & Gruson, D. (2020) A biological profile for diagnosis and outcome of COVID-19 patients. *Clin. Chem. Lab. Med*., **58**, 2141-2150.

Long, H., Nie, L., Xiang, X., Li, H., Zhang, X., Fu, X., Ren, H., Liu, W., Wang, Q. & Wu, Q. (2020) D-dimer and prothrombin time are the significant indicators of severe COVID-19 and poor prognosis. *Biomed. Res. Int*., **2020**, 6159720.

Zheng, J. (2020) SARS-CoV-2: an emerging coronavirus that causes a global threat. *Int. J. Biol. Sci*., **16**, 1678-1685.