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Author (s):	Maria Bibi[1], Braira Wahid[2], Syed Sib Tul Hassan Shah[3]
Affiliation (s):	[1]University of Management and Technology (UMT), Lahore Pakistan [2]Monash University, Clayton, Victoria, Australia [3]Zhejiang Sci-Tech University, Hangzhou, China
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Molecular Epidemiology of SARS-CoV-2 and Correlation of its Clinical Severity with Different Biochemical Parameters: A Retrospective Study

Maria Bibi[1+], Braira Wahid[2+], Syed Sib Tul Hassan Shah[3*+]

[1]Department of Life Science, School of Science, University of Management and Technology (UMT), Lahore, Pakistan
[2]Laboratory of Antimicrobial Systems Pharmacology, Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, Victoria, Australia
[3]Zhejiang Province Key Laboratory of Plant Secondary Metabolism and Regulation, College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, China

ABSTRACT

Background Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is an infectious disease that was first identified in December 2019 in Wuhan, the capital of China's Hubei province. Since then, it has spread globally, resulting in the ongoing SARS-CoV-2 pandemic. In Pakistan, over 1.5 million cases have been reported since February 2020 (when the first case was reported).

Method This retrospective study was conducted by classifying the data of 136 patients into three study groups, namely asymptomatic (n = 84), mild (n = 36), and severe (n = 16). The data was analyzed using IBM SPSS (version 21).

Results Age and gender showed a non-significant relationship with SARS-CoV-2. Biochemical markers namely D-DIMER, FERRITIN, CRP, and PT showed significant results with p-values 0.001, 0.001, 0.048, and 0.009, respectively (p<0.05). On the contrary, APTT showed a non-significant relationship with SARS-CoV-2 (p= 0.146).

Conclusion It was concluded that the biochemical parameters have seen as the best prediction markers to gauge the SARS-CoV-2 infection severity. Furthermore, this research established the correlation of biochemical parameters with SARS-CoV-2 infection severity and also highlighted the use of these biomarkers as diagnostic and therapeutic biomarkers.

Keywords: Activated Partial Prothrombin Test (APTT), C-Reactive Protein (CRP) test, Middle East respiratory syndrome coronavirus (MERS-CoV), Prothrombin Test (PT), Severe Acute Respirator Syndrome Coronavirus 2 (SARS-CoV-2), biochemical markers

[+]All authors contribute equally
[*]Corresponding Author: hassanshahsibtul@gmail.com

Highlights

- Most of the infected candidates were asymptomatic hence showed no symptoms, and there is a direct relation of the infection with the age of the people. People with age > 40 were more prone to the SARS-COV-2 infection.
- There is a significant correlation between SARS-COV-2 infection and variations in biochemical markers such as D-Dimer, CRP, Ferritin, and PT.
- D-Dimer, CRP, Ferritin, and PT were identified as predictive biomarkers for the severity of the SARS-COV-2 infection. Elevated levels of these markers in the serum are directly associated with the severity of the infection.

1. INTRODUCTION

In December 2019, various cases of pneumonia of a mysterious etiology were reported in Wuhan, Hubei province, China and suddenly caused a serious public health hazard [1]. The symptoms appeared in most of the patients and some of them were rapidly exposed to Acute Respiratory Distress Syndrome (ARDS) and acute respiratory failure [2]. In early January 2020, a novel virus was recognized from an infected patient using next-generation sequencing by the Chinese Center for Disease Control and Prevention. It was formally named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Meanwhile, the disease was named as Coronavirus Disease 2019 (SARS-CoV-2) by the World Health Organization (WHO).

Coronaviruses are enveloped positive-stranded RNA viruses [3]. Full-genome sequencing and phylogenic analysis indicated that the coronavirus that causes SARS-CoV-2 is a beta coronavirus in the same subgenus as the Severe Acute Respiratory Syndrome (SARS) virus, but in a different clade. In the family of coronaviruses, the recently identified SARS-CoV-2 is on the 7th number and resembles the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) [4]. Consensus held that the novel coronavirus was transferred to human beings from a bat after it was eaten by one of the inhabitants of Wuhan.

COVID-19 became an epidemic in a number of days. The WHO announced an alarming situation and declared it a global pandemic [5]. This disease was transferred through human-to-human interaction. There were different symptoms seen in SARS-CoV-2 infected patients. Some of them were mild and various patients remained asymptomatic. Major symptoms seen in different patients included fever, dry cough, tiredness, aches, pain, and loss of taste and smell. The sequence of genome of SARS-CoV-2 was almost 88% similar to bat- SL-CoV-ZC45 and was 96.2% similar to bat CoV-RaTG13 [6].

SARS-CoV-2 spread globally and recent updates by the WHO revealed that over 698.03 million cases have been reported [7]. The source of transmission is close contact with each other, at a distance of less than six feet. A previous study showed that coronaviruses can be the cause of severe respiratory and intestinal infections. Before the outbreak of SARS in 2002 and 2003 in Guangdong, China, coronaviruses generally were not considered as highly pathogenic [8]. The Middle East respiratory syndrome coronavirus (MERS-CoV) appeared in the Middle Eastern countries in 2012. It was a highly pathogenic coronavirus, not only causing infection but also due to its

mortality and mobility rate [9]. Its epidemiological trends in different countries have been studied.

In Pakistan, the first case of COVID-19 was reported on January 25, 2020 [7]. Afterward, the number of cases started to increase. Even in May 2021, some Asian countries were still experiencing a terrible third wave of this pandemic. SARS-CoV-2 has been associated with different biochemical and hematological parameters, such as D-DIMER, CRP, PT, APTT used to measure hemoglobin, hematocrit (HCT), FERRITIN, RBCs, and WBCs [10]. D-DIMER, PT, and APTT tests are performed to check whether the blood is clotting or normal, while FERRITIN is performed to check the iron content in the patient's body and CRP is performed to check any inflammation [11]. The current study establishes the correlation between the severity of SARS-CoV-2 and FERRITIN, D-DIMER, PTT, APTT, and CRP. It is aimed to determine the epidemiological trends of SARS-CoV-2 in Lahore, Pakistan to analyze the correlation of different hematological parameters, namely FERRITIN, PT, APTT, and D-DIMER, with the severity of SARS-CoV-2 infection, and to establish the link between clinical severity and the C-Reactive Protein (CRP).

2. MATERIALS AND METHODS

2.1. Study Design

The current study analyzes the biochemical parameters of SARS-CoV-2 and their correlation with clinical biomarkers namely C-Reactive Protein (CRP), FERRITIN, D-DIMER, PTT, and APTT. The data was collected from the Genome Center for Molecular Based Diagnostics and Research Center (GCMDR), Lahore, Pakistan Kidney and Liver Institute (PKLI), General Hospital Lahore, and Citi Lab and Research Center (CRC) from November 2020 to February 2021. The patients were classified in three study groups, namely asymptomatic, mild, and severe on the basis of the severity of their symptoms.

2.2. Data Analysis

Data analysis was performed by using IBM SPSS (version 21). The tests performed to analyze the data were t-test, chi square test, ANOVA, and Spearman's correlation. The analysis of epidemiological trends and all variables was done and mean and standard deviation (SD) were calculated. Statistical differences were calculated using chi-square/t-test. Spearman's correlation coefficient was used to determine the relationship among different variables. Data was analyzed based on different categories including age, gender, clinical biomarkers, comorbid conditions, as well as by analyzing the correlations of clinical biomarkers and clinical severity.

3. RESULTS

A total of 136 patients were enrolled in this study. The correlation of SARS-CoV-2 with different clinical parameters was analyzed by grouping the patients into three categories based on symptoms that appeared during the course of infection. Of the selected patients, a total of 84 patients were asymptomatic, 36 were mildly infected, and 16 were severely infected.

3.1. Gender Wise Distribution of Participants among Different Study Groups

In this study, the participants were further categorized on the basis of gender. In total, there were 92 (67.6%) male and 44 female participants (32.35%). The asymptomatic study group consisted of a total of 84 asymptomatic participants, of

which 52 (62%) were male and 32 (38%) were female. The mild study group had a total of 36 mildly infected participants, of which 27 (75%) were male and 9 (25%) were female. The severe study group had a total of 16 severely infected participants, of which 13 (81.25%) were male and 3 (11.75%) were female. The prevalence of male participants among all study groups was significantly higher as compared to female participants. Still, a non-significant relationship (p=0.19) was observed between gender and various study groups.

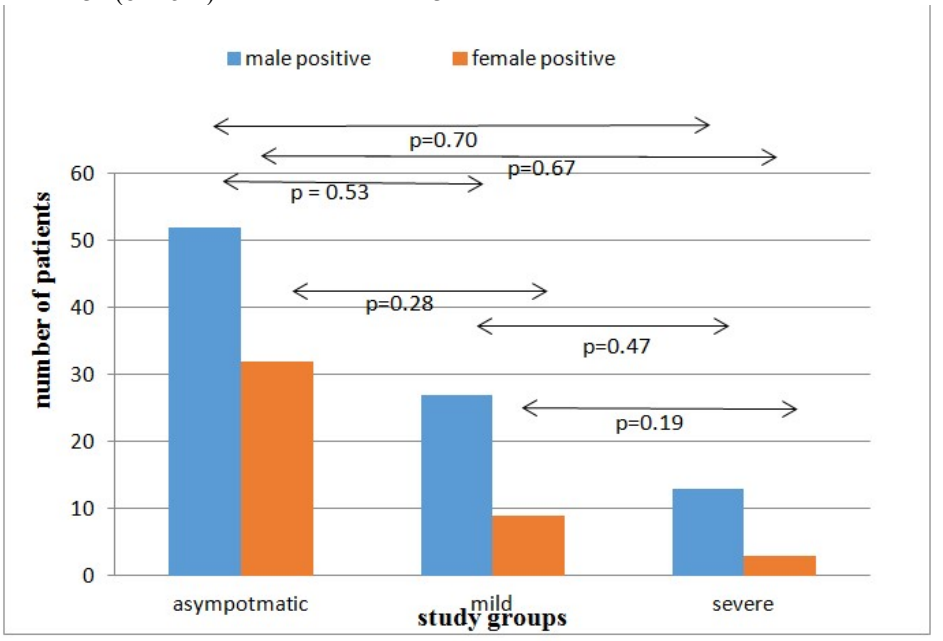

Figure 1. Graphical Representation of Gender Wise Distribution of Data

3.2. Age Wise Distribution of Participants among Study Groups

In this study, the patients were divided into five age groups, that is, 0-20, 21-40, 41-60, 61-80, and 81-100. Then, each age group was further divided into study groups, namely asymptomatic, mild, and severe. The median age for the patients was 54.54±18.76. The findings showed that the highest prevalence of disease was in asymptomatic patients with the mean age 53.02±19.37, while the lowest prevalence was in severe patients with the mean age 58.13±13.67. The patients' mean age in the mild study group was 56.47±19.37. The majority of the patients were in their middle age, that is, 41-60. Sever cases were the highest in the age group 61-80 with 50% prevalence. Asymptomatic and mild study groups showed the highest prevalence in the case of middle age group (41-60) with the prevalence ratio of 33.33% and 38.89%, respectively.

3.3. Gender Wise Distribution of Participants among Age Groups

The findings showed the highest prevalence in male patients of the age group 41-60. In case of female patients, the age group 61-80 had the highest prevalence.

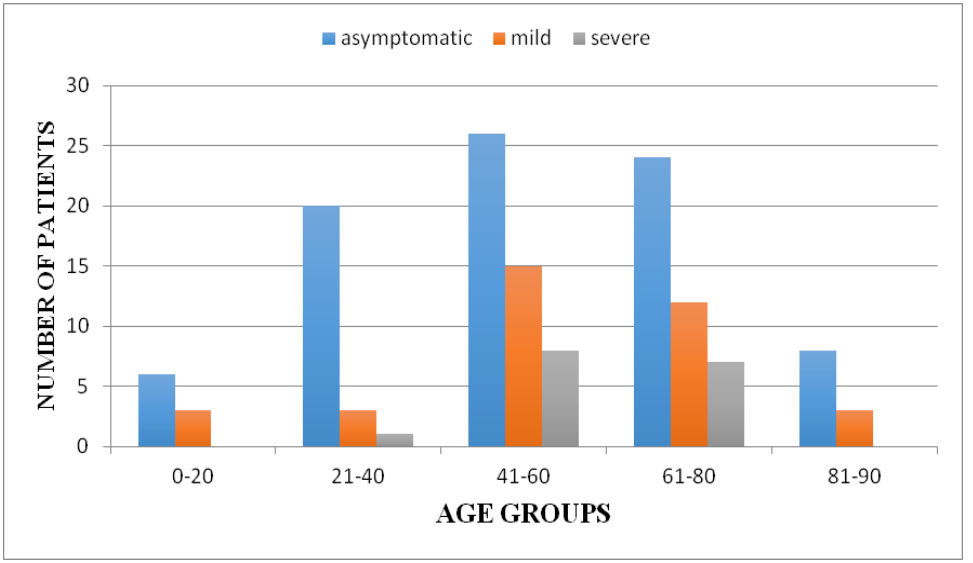

Figure 2. Graphical Representation of Age Wise Distribution

Table 1. Overall Mean Values of All Clinical Parameters

SR. NO	Clinical Severity	FERRITIN (ng/ml)	CRP (mg/ml)	PTT (seconds)	APTT (seconds)
		Mean±S.D	Mean±S.D	Mean±S.D	Mean±S.D
1	Asymptomatic	614.79±544.14	3.96±4.78	11.73±4.31	30.30±7.00
2	Mild	1080.28±608.39	4.56±4.29	13.95±3.98	34.14±16.72
3	Severe	1847.75±579.71	7.16±6.08	14.12±3.21	33.74±9.23

3.4. Clinical Biomarker Effects in Study Groups

The biomarkers FERRITIN, CRP, PTT, APTT, and D-DIMER were accounted for in this study to check their correlation with disease condition. Their values indicated the severity of SARS-CoV-2 infection. The mean values of FERRITIN, CRP, and PTT were higher in severe condition, which shows that the higher values of these biomarkers are indicative of the severity of the disease. These biomarkers showed the highest value in severe patients, as compared to mild and asymptomatic patients. In case of APTT, the higher mean value was of the mild study group, rather than severe and asymptomatic study groups. Asymptomatic patients mostly showed normal values of this biomarker.

3.5. Correlation of Biomarkers with Study Groups

The findings indicated that the selected biomarkers are significantly correlated with the clinical severity of SARS-CoV-2 infection.

3.5.1. D-DIMER. ANNOVA test was applied for the three study groups. The results of D-DIMER were in categorical form; therefore, they were put in a variable form in which 1 stood for positive and 2 stood for negative. A significant relationship was found between D-DIMER

and the respective disease condition (p=.001). The graph (Figure 1-3A) clearly indicates the positive value of D-DIMER in severe and mild conditions and a negative value in asymptomatic conditions. The F-value is higher, which shows the higher variability in the sample in case of D-DIMER (F=152.5).

3.5.2. FERRITIN (ng/ml). FERRITIN (ng/ml) showed a significant relationship with the clinical severity of the disease (p=.001). The F-value is higher which shows the higher variability between the groups (F=38.7). A direct relationship exists between the clinical severity of SARS-CoV-2 infection and FERRITIN (ng/ml). The values of FERRITIN (ng/ml) were found to be higher in patients with severe and mild symptoms but almost normal in asymptomatic patients. The graph (Figure 1-3B) gives the perfect interpretation for FERRITIN values that are higher in patients with severe symptoms and lower in mild to asymptomatic patients.

3.5.3. CRP (mg/ml). A direct relation was observed between clinical severity and C-Reactive Protein (CRP). They showed a significant relationship with each other (p=.048), with a high value of F-test (F=3.11) and lesser variability between the groups. CRP value was found to be higher in patients with a severe condition of SARS-CoV-2 infection. Similarly, a higher value of CRP was observed in mild patients, while its value was almost normal in asymptomatic patients, although it may have increased with the further progression of the disease. The line in graph (Figure 1-3C) shows how the values of CRP decrease from severe to asymptomatic patients, showing a direct relationship between them.

3.5.4. APTT (sec). Clinical severity and APTT showed a non-significant relationship (p= .146) with little variance in study groups (F=1.93). Clinical severity in the three study groups, namely severe, mild, and asymptomatic. APTT, partial thromboplastin time test kept at y-axis and the clinical severity at x-axis as independent variable. It showed a direct relationship between clinical severity and coagulation test. Similarly, line progression from asymptomatic patients to severe patients shows the increase in APTT values from asymptomatic patients to severe patients (Figure 3D).

3.5.5. PTT (sec). Prothrombin Time Test (PTT) showed a significant relationship with clinical severity (p=.009). The F-test value was 4.917 which showed slight variance among the study groups. Clinical severity showed a direct and significant relationship with PTT values (Figure 3E).

3.6. Correlation of CRP (mg/ml) with FERRITIN (ng/ml), PTT (sec) and APTT (sec)

C-Reactive Protein (CRP) is a biomarker that increases when the SARS-CoV-2 infection progresses. Spearmen's correlation was used to check whether this biomarker has any effect over other clinical biomarkers or not. This study observed that FERRITIN and APTT did not show any significant correlation with CRP, as the significance value of Spearman's correlation was greater than the significance value (p=0.05). CRP and FERRITIN showed a correlation coefficient value of 0.100 which depicts a non-significant relation. The value of Spearman's correlation coefficient of CRP and APTT was 0.137, which also showed a non-significant correlation between both clinical parameters. Their significance values showed that they had no effect over each other. It indicates that if CRP changes,

then it is not necessary that clinical biomarkers also change. The Spearman's correlation coefficient value for CRP and PTT was 0.018, which is smaller than the significance value. It indicates that they affect each other. Hence, if CRP increases during SARS-CoV-2 infection, then PTT also shows a significant increase in its values.

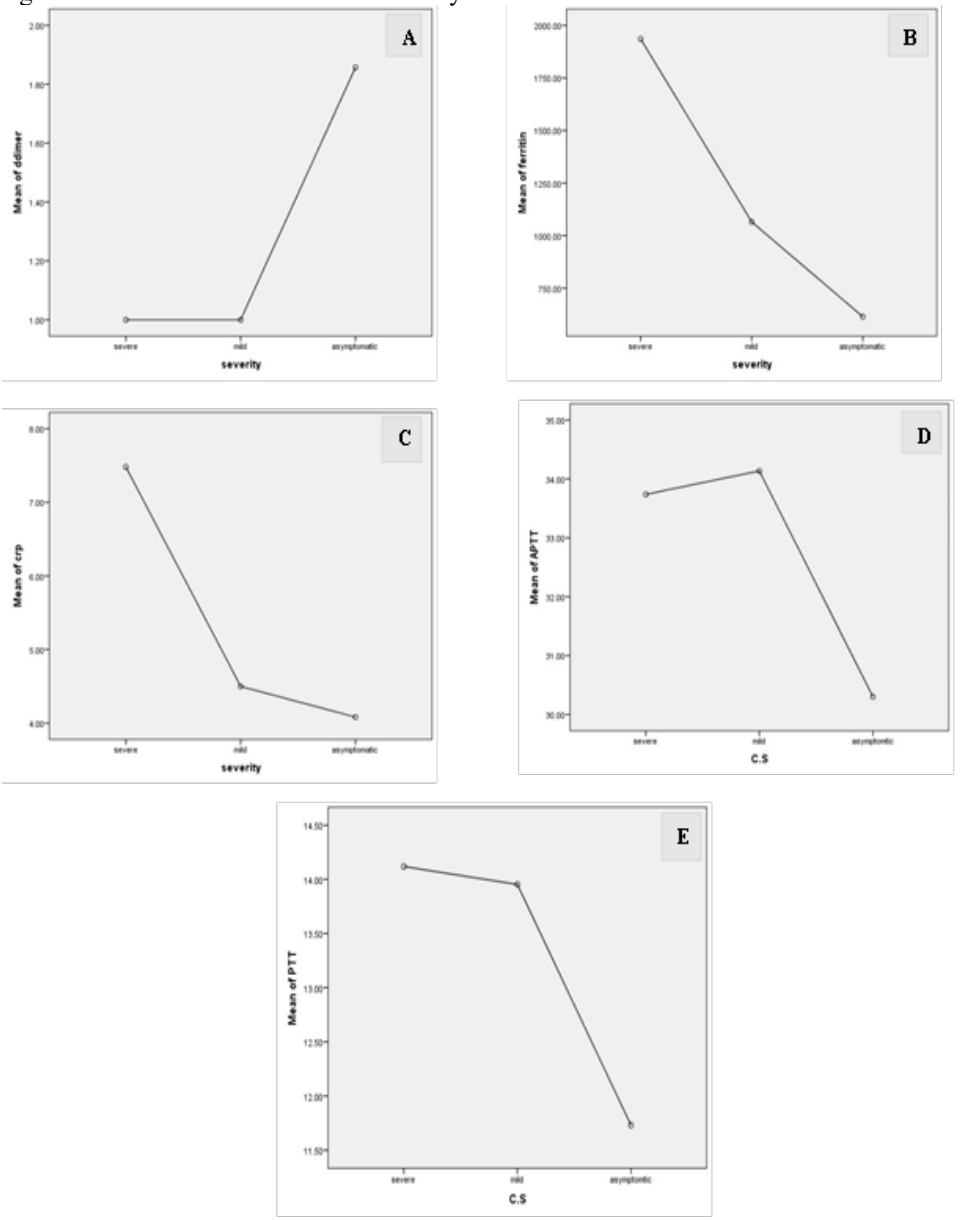

Figure 3. Graphical Representation of the Correlation of Clinical Parameters with Severity

Table 2. Spearman's Correlation between CRP and Other Clinical Parameters

Sr. no.	Correlation variables	Correlation of coefficient/spearman's rho(rs)	p value
1	Clinical severity/CRP		p= 0.048
2	Clinical severity/Ferritin		p= 0.001
3	Clinical severity/D-dimer		p= 0.001
4	Clinical severity/PT		p= 0.009
5	Clinical severity/aPTT		p= 0.146
6	CRP/Ferittin	0.100	p= 0.100
7	CRP/PT	0.018	p= 0.018
8	CRP/aPTT	0.137	p= 0.137

4. DISCUSSION

The findings didn't show any significant relation of the disease with age. Retrospectively, the current study showed the highest prevalence rate in the middle age group, that is, 41-60, with 36.02% prevalence. SARS-CoV-2 affects the individuals equally. Although, some studies have mentioned that children, diseased, and older people have more chances to be infected as compared to healthy adults.

The inflammatory biomarker D-DIMER showed a significant correlation with the severity of the disease. The biomarker was higher in patients with severe and mild conditions, while none of the patients was tested positive for D-DIMER among asymptomatic patients. This may be due to the fact that at the initial stage of the infection D-DIMER remained normal. However, with the worsening of the disease it became positive. A retrospective study [12] also described that D-DIMER has a significant relationship with the severity of the disease. The results clearly mentioned that the level of D-DIMER in the patients increased with the severity of clinical outcomes of SARS-CoV-2.

A small number of studies showed that FERRITIN does not have any significant relationship with SARS-CoV-2 [13]. In another study, the levels of serum FERRITIN were noted to be much higher in the non-severe group of patients, as compared to the results of this study. On the contrary, the association between the levels of FERRITIN and disease severity showed a significant correlation in the current study. Another study showed similar results where the elevated levels of FERRITIN were seen in severe hypoxemia but not in relation with SARS-CoV-2 [14].

The coagulation biomarkers PTT and APTT also act as the biomarkers that must be tested at the initial stages of COVID-19 infection. A number of studies showed a significant positive correlation between APTT and clinical severity, although in the current study the relation with APTT was found to be insignificant [15]. On the contrary, the correlation between PT and clinical severity was found to be significant. Similar results were noted in a number of studies. Coagulation factors have a critical role in diseases. Hence, the level of PT increases with the severity of the disease.

The main parameter, that is, CRP showed an elevated level in a large number of studies, similar to the current findings.

The result was consistent with the findings of a review article which concluded that the inflammatory biomarker CRP amounted to 60.7% of patients infected with SARS-CoV-2 and was a crucial marker for predicting SARS-CoV-2 prognosis and mortality in these patients [16]. The current analysis confirmed a significant elevation in CRP among severely infected patients, while the concentration was normal in asymptomatic patients. The values of CRP increased during the infection as it proceeded from asymptomatic to severe. Therefore, severe patients showed the highest elevated values of CRP that identified it as a potential biomarker for the indication of SARS-CoV-2 severity. To conclude, it is a nonspecific and momentous marker of inflammation that shows a direct correlation with SARS-CoV-2.

The findings observed the existence of a significant mutual relationship between clinical biomarkers and clinical severity. With the progression of infection, the values of these biomarkers that are symptomatic of the Acute Respiratory Syndrome (ARDS) increase and result in the disfunctioning of organs. Therefore, it was concluded that the abnormal values of these biochemical markers are indicative of the severity of the disease and help to develop drugs and medicines for its treatment, accordingly.

4.1. Clinical Significance

- Due to a small sample size and pandemic/lockdown, there is a need to conduct a similar research based on a large population size.
- The correlation of biochemical parameters (CRP, D-DIMER, FERRITIN, PT, and APTT) with clinical severity was established in this study. Hence, there is a need to conduct further research on the underlying mechanism behind this correlation.
- The current study also highlighted the use of the above-mentioned biochemical biomarkers as diagnostic and therapeutic biomarkers.

4.2. Conclusion

The current study observed the high prevalence of COVID-19 among male patients in the middle age group. The findings highlighted a non-significant relationship of age and gender with clinical severity. The prevalence was 61.77% in asymptomatic patients, 26.47% in patients with mild symptoms, and 11.76% in severe cases. The selected biomarkers (D-DIMER, CRP, FERRITIN, PT, and APTT) showed a significant correlation with clinical severity. They showed elevated values in severe and mild cases of COVID-19. PT and FERITIN showed a significant relationship with CRP, while APTT did not. Such findings are critical and can also be used as guidelines to assess the clinical severity of the SARS-CoV-2 infection and to treat the patients suffering with this disease.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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