Original Article

# Impact of Inflammatory Biomarkers and Cycle Threshold (Ct) Values on the Progression of COVID-19 Disease in Iraq—Wasit Governorate

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## Abstract

**Background:** The relationship between the severity of COVID-19 disease and key biomarkers such as D-dimer, C-reactive protein (CRP), lactate dehydrogenase (LDH), and ferritin, and the neutrophil-to-lymphocyte ratio (NLR), as well as their association with cycle threshold (Ct) and blood group, is important to understand thoroughly for managing COVID-19. **Objectives:** This study aimed to measure the levels of these biomarkers and their relationship to the other clinical data of patients. **Materials and Methods:** A total of 229 collected specimens including 45 severe to critical cases, 45 mild to moderate cases, as well as 50 recoveries, and 89 healthy control groups were diagnosed using real-time polymerase chain reaction, and a blood test was performed using an automated analyzer. **Results:** The results showed that older individuals and those with higher weights are more susceptible to developing severe COVID-19 infections, whereas younger age groups and individuals with lower weights are more likely to recover from this disease. Furthermore, the study found no clear association between the COVID-19 virus and fluctuations in hemoglobin levels. However, significant statistical increases were observed in two patient groups compared to the recovered group and the reference ranges for white blood cell count, NLR, and concentrations of CRP, ferritin, D-dimer, and LDH. **Conclusions:** Elderly individuals and those with chronic medical conditions may exhibit higher viral loads (measure by Ct values) that correlate positively with elevated levels of CRP, D-dimer, LDH, and ferritin concentrations. However, it is important to note that this study does not emphasize blood type as a predictive model for COVID-19 disease.

**Keywords:** COVID-19, CRP, D-dimer, ferritin, LDH

## Introduction

The World Health Organization launched the term COVID-19 and declared this novel coronavirus disease, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as a pandemic on March 11, 2020. The virus is highly contagious, and the incubation period ranges between 2 and 14 days.[1] Many infected people are either asymptomatic or develop a mild respiratory illness, progressing to severe conditions such as pneumonia or acute respiratory distress syndrome, with higher mortality rates among the elderly and patients with comorbidities.[2] Iraq is among the countries with the highest number of coronavirus disease (COVID-19) cases. The first confirmed case of COVID-19 in Iraq was reported on February 24, 2020, in Najaf Governorate, involving an Iranian student who had traveled to Iran. Subsequently, four cases were reported within one family in Kirkuk Governorate on February 25, all of whom had traveled to Iran. On February 27, a new case was reported in Baghdad, involving a patient who had also recently visited Iran. As of March 12, 2020, 74 confirmed cases and 8 deaths had been reported across Iraq. On April 16, 2020, the number of confirmed cases grew to 1415, with 78 deaths. COVID-19 instances had reached 4469 by May 24, 2020, with 160

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deaths and 2738 individuals having recovered from the virus.[3]

The pathogenic characteristics are largely dependent upon the nature of the genes residing in the organism's genome.[4] Biomarker changes and high rates of thrombotic complications have been reported in patients with severe COVID-19.[5] Circulating inflammatory biomarkers [ferritin, lactate dehydrogenase (LDH), and D-dimer] representing the immune system and inflammation have been considered prognostic indicators.[6] Higher levels of D-dimer, serum ferritin, and LDH were associated with older age and the severity of COVID-19.[7] Also, C-reactive protein (CRP) titer, lymphocyte, and neutrophil count are important parameters.[8] Therefore, this study aims to clarify the role of these inflammatory markers and some hematological factors that can help in obtaining a deeper understanding of the mechanism of pathogenesis of this disease, because the relationship between these inflammatory markers (CRP, ferritin, LDH, and D-dimer) as well as complete blood count (CBC) and cycle threshold (Ct) with SARS-CoV-2 RNA is still unclear.

## Materials and Methods

### Populations studied

The current case-control study involved 229 blood samples collected from Al-Shifa Center in Al-Zahraa Teaching Hospital in Kut and from different parts of Wasit Governorate from November 2022 to March 2023. These samples were obtained from patients at varying degrees of COVID-19 disease severity (measured by the degree of organ system disruption or physiologic decompensation). Additionally, samples were collected from individuals who had recovered 3 weeks after being diagnosed positive with quantitative reverse transcription polymerase chain reaction (qRT-PCR) at Al-Karama Teaching Hospital in Kut City, as well as from people who did not fall ill and were not vaccinated. The practical part of the study was completed at Kut National Central Laboratory for Public Health, which included performing a CBC and assessing CRP levels, LDH, D-dimer, and blood ferritin tests, as well as measuring their blood sugar levels and determining the presence of high blood pressure. The study also included IgG and IgM tests and blood type determination for all study groups. According to WHO recommendations for COVID-19 infections, instances were classified as mild-moderate, severe-critical, and recovered groups,[9] depending on physician's detection and recovery times. This study included 45 mild-moderate cases, 45 severe-critical cases (including 6 deaths), 50 recovered cases, and 89 control cases. Body mass index (BMI), sex, and age groups were also included in this research.

The inclusion criteria included that all recovered COVID-19 samples must have previously been infected with COVID-19 and recovered from this disease. The results of the qRT-PCR test, as well as IgM, must be negative, while the IgG test result must be positive for the COVID-19 virus. In the healthy cases, who are apparently healthy, their COVID-19 antibodies (IgM and IgG) and qRT-PCR results were negative. Meanwhile, in the mild-moderate and sever-critical groups, their COVID-19 antibodies (IgM) and the qRT-PCR were positive. The exclusion criteria included, deleting samples within the same family and samples less than 10 years old. The ages of the individuals in this study ranged from 20 to 90 years, and they were 99 males and 130 females.

### Laboratory tests

Nasopharyngeal (NP) swabs, together with 5 mL of blood samples, were collected from the study groups. The swabs were placed directly in viral transport media. The viral RNA was isolated by Mini kit QIAamp Viral RNA. SARS-CoV-2 was diagnosed by qRT-PCR using a commercial kit and followed by the manufacturer's instructions.

The blood samples of the groups under the current study were divided into three tubes [sodium citrate tubes, gel tubes, and ethylenediaminetetraacetic acid (EDTA) tubes], and the first part of the blood samples (sodium citrate tubes) was centrifuged for 20 min at 3500 rpm in order to obtain plasma, which was later used for the purpose of determining plasma D-dimer analysis using a specific automated protein analyzer (BIOSENCER STANDARD F200 analyzer) provided by (Suwonsi, Gyeonggi Co., Ltd., Suwon, Gyeonggi Province, South Korea). Plasma samples of all patients were fed into this device, and then the D-dimer concentration was automatically calculated. The second part of the blood samples (gel tube) was centrifuged for 10 min at 6000 rpm to obtain serum used to determine blood sugar, CRP titer, LDH, and ferritin. Serum concentrations of LDH and CRP were evaluated using electro-chemiluminescence immunoassay, Roche Cobas Integra 400 plus (Roche Diagnostics GmbH, Mannheim, Germany). At the same time, the ferritin and antibodies were assessed using a miniVIDAS analyzer (enzyme-linked fluorescent assay; (BioMerieux, Marcy-l'Étoile, France). Serum samples for all patients were applied to the instrument, and then the concentration of LDH, CRP, and ferritin was automatically calculated. A third portion of blood samples collected in EDTA tubes was used for blood group determinations and also for complete blood counting by an automated hematology analyzer. The neutrophil-to-lymphocyte ratio (NLR) is found by dividing the neutrophil absolute by the lymphocyte absolute.[10]

### Statistical analysis

Number and percentage were used to describe categorical variables, and significant differences were assessed by a two-tailed Fisher exact test. Continuous variables were tested for normality using the Kolmogorov–Smirnov

**Table 1: Baseline characteristics of the COVID-19 patients group, the recovered group, and the healthy control group**

| Characteristic | | Patient ($n$ = 90) | Recovered ($n$ = 50) | Healthy control ($n$ = 89) | $P$ value |
|---|---|---|---|---|---|
| Age (year) | | 47.9 ± 17.8 | 38.1 ± 14.7 | 37.2 ± 14.1 | **<0.01** |
| Age group | ≤45 | 46 (51.11) | 33 (66) | 45 (50.56) | 0.16 |
| | >45 | 44 (48.89) | 17 (34) | 44 (49.43) | |
| Sex | Male | 42 (46.67) | 21 (42) | 46 (51.68) | 0.53 |
| | Female | 48 (53.33) | 29 (58) | 43 (48.31) | |
| Body mass index | Normal weight | 29 (32.22) | 27 (54) | 44 (49.43) | **0.006** |
| | Overweight/obese | 61 (67.78) | 23 (46) | 45 (50.56) | |
| Blood group | A | 27 (30) | 15 (30) | 23 (25.84) | 0.099 |
| | B | 20 (22.22) | 12 (24) | 23 (25.84) | |
| | O | 31 (34.44) | 15 (30) | 21 (23.59) | |
| | AB | 12 (13.33) | 8 (16) | 22 (24.71) | |
| Diabetes mellitus | Yes | 31 (34.44) | 6 (12) | 19 (21.34) | **0.009** |
| | No | 59 (65.55) | 44 (88) | 70 (78.65) | |
| Hypertension | Yes | 28 (31.11) | 5 (10) | 14 (15.73) | **0.003** |
| | No | 62 (68.89) | 45 (90) | 75 (84.27) | |

Values of age are given as mean with standard deviation (continues variables) or number and percentage (categorical variables). *P*: probability of ANOVA test (to compare continues variables), Pearson Chi-square test (to compare categorical variables). Significant *P* value is indicated in bold

and Shapiro–Wilk tests. Normally distributed variables were given as mean and standard deviation (SD), and significant differences were evaluated using the Student *t* test. Nonparametric variables (skewed) were expressed as the median and interquartile range (IQR), and the Mann–Whitney *U* test was used to determine significant differences. The kind and degree of the association between variables were explained using Pearson's correlation (*R*). This analysis was carried out using the statistical tool SPSS version 25.0 (IBM Corp., Armonk, New York) and GraphPad Prism version 8.0 (GraphPad Software, San Diego, California ).

## Results

### Baseline characteristics of the COVID-19 patients group, the recovered group, and the healthy control group

The basic characteristics of the COVID-19 patients group ($n$ = 90), the recovered group ($n$ = 50), and the healthy control group ($n$ = 89) were studied to understand the relationships between these three groups. Significant differences were observed in the ages of these three groups (47.9 ± 17.8 in the patients group, 38.1 ± 14.7 in the recovered group, and 37.2 ± 14.1 in the healthy control group) that were studied ($P$ < 0.01). On the other hand, significant differences were also noted regarding BMI, diabetes, and hypertension, with *P*-values of 0.006, 0.009, and 0.003, respectively, among the study groups. It was noted that 67.8% of the patients were overweight/obese compared to 32.2% with normal weight in patient group based on BMI. Additionally, the percentages for diabetics were 34.44%, 12%, and 21.34% in the patients group, recovered group, and healthy control group, respectively. For non-diabetics, the percentages were 65.55%, 88%, and 78.65% in patients group, recovered group, and healthy controls group, respectively, regarding diabetes mellitus. Similarly, the percentages for hypertensive patients were 31.11%, 10%, and 15.73%, and for non-hypertensive individuals, they were 68.89%, 90%, and 84.27% in patients group, recovered group, and healthy controls group, respectively, regarding hypertension.

While with regard to the age groups ≤45 years, and the age groups older than that, sex, blood groups and Ct values, no significant differences were observed between those groups [Table 1].

### Hematological parameter

When studying baseline blood parameters in COVID-19 patients and the recovered group with the reference range for these parameters, no significant differences were observed in hemoglobin concentrations ($P$ = 0.548), while there were significant differences ($P$ < 0.001) regarding white blood cell (WBC) count, NLR, and concentrations of CRP, ferritin, D-dimer, and LDH. The numbers, ratios, and concentrations of all these parameters in the group of patients with severe to critical conditions were higher than in the group of patients with mild to moderate conditions, and also higher than in the group of recovered individuals [Table 2].

On the other hand, when studying the concentration of the key classical parameters such as CRP, ferritin, D-dimer, and LDH separately for COVID-19 patients stratified by age group/year (>45 and ≤45 years), sex, BMI, disease severity, and Ct values, it was noted that there were significant differences for each of the age, disease severity, and Ct values for each of these parameters, which indicates the existence of a strong correlation between them, while no statistically significant differences were observed for both sex and BMI for each of them [Figure 1].

**Table 2: Baseline blood parameters in COVID-19 patients' group and recovered group**

| Parameter | COVID-19 patients ($n$ = 90) | | Recovered ($n$ = 50) | Reference range[a] | $P$ value |
|---|---|---|---|---|---|
| | Mild-moderate ($n$ = 45) | Severe-critical ($n$= 45) | | | |
| Hemoglobin (g/dL) | 11.5 (11.1–14.6) | 14.3 (11.1–15.4) | 12.2 (11.2–15.6) | 11.6–16.6 | 0.548 |
| WBC (× $10^9$/L) | 11.1 ± 0.86 | 12.2 ± 2.5 | 6.3 ± 2.02 | 3.4–9.6 | **<0.001** |
| NLR | 4 (3.8–4.2) | 6.1 (4.7–6.9) | 2 (1.8–2.3) | 1.8–2 | **<0.001** |
| CRP (mg/L) | 16.9 (14.7–20.8) | 45.5 (40.7–49.3) | 4.5 (3–5) | 8–10 | **<0.001** |
| Ferritin (ng/mL) | 390.8 (369.4–450.8) | 915.2 (775.2–987.8) | 98.3 (82.5–188.1) | 20–250 | **<0.001** |
| D-dimer (mg/L) | 416.3 (196.8–483.5) | 4012.4 (1225.5–6582.3) | 65.8 (44.8–105) | ≤500 | **<0.001** |
| LDH (IU/L) | 342.3 (285.4–390.6) | 873.2 (745.4–906.4) | 166 (154.8–168.3) | 105–333 | **<0.001** |

Data are given as mean ± SD, median, and IQR (continuous variables) or number and percentage (categorical variables as present). Means ± SD were compared with the analysis of variance test. Medians were compared with the Kruskal–Wallis test. Significant $P$ value is indicated in bold

WBC: white blood cell; NLR: neutrophil-to-lymphocyte ratio; CRP: C-reactive protein; LDH: lactate dehydrogenase; SD: standard deviation; IQR: interquartile range

[a]Data source from https://www.mayoclinic.org

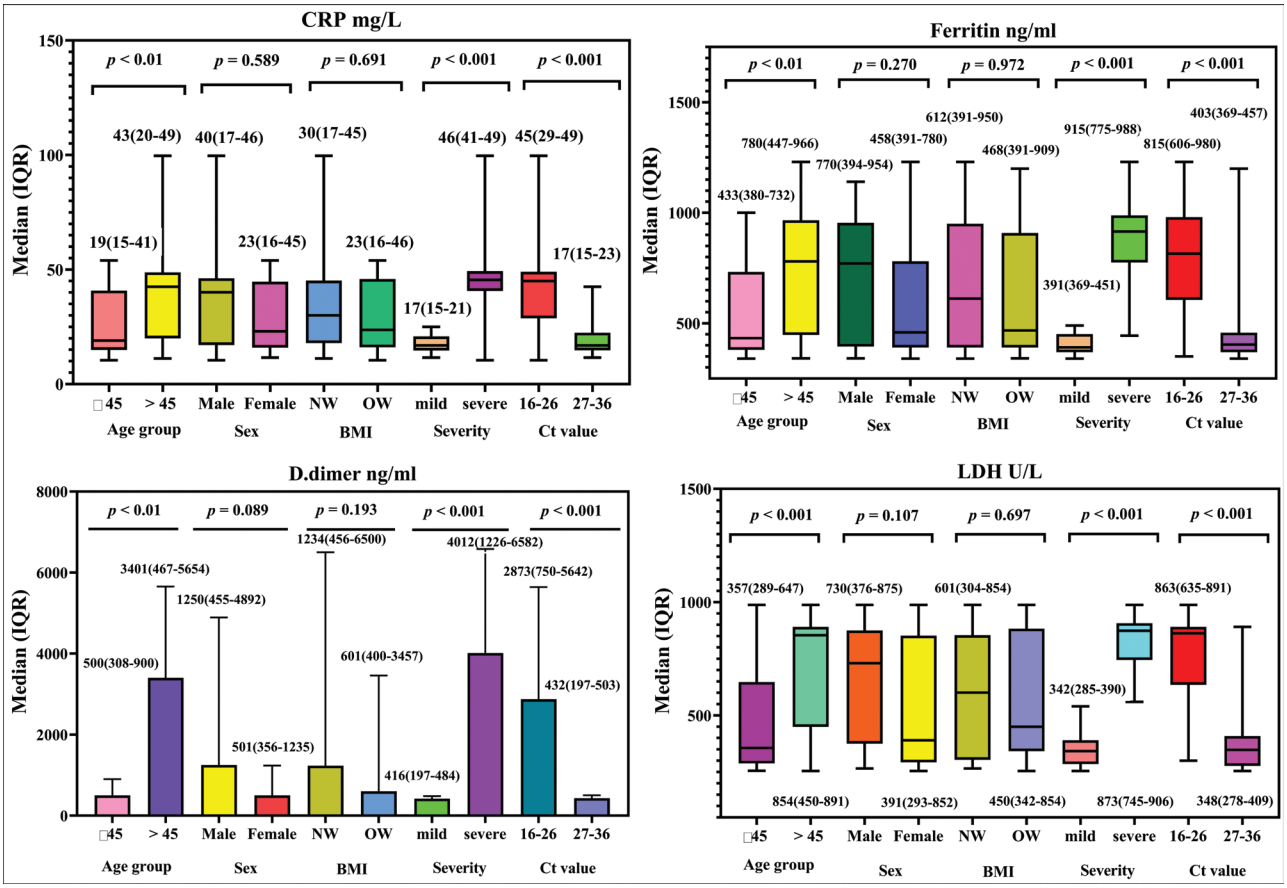


**Figure 1:** Box plot of CRP, ferritin, D-dimer, and LDH concentration stratified by age group/year (≤45 and >45 years), sex (male and female), BMI, severity, and Ct value in COVID-19 patients. CRP: C-reactive protein; LDH: lactate dehydrogenase; BMI: body mass index; NW: normal weight, OW: overweight/obese; $p$: probability of Mann–Whitney $U$ test (to compare continuous variables)

On the other hand, when studying the frequency of Ct values 16–26 that represent high viral load and values 27–36 that represent low viral load in COVID-19 patients stratified by age, disease severity, chronic diseases, BMI, and blood groups. There were statistically significant differences with regard to age ($P$ = 0.02), disease severity ($P$ < 0.001), and chronic diseases (diabetes and hypertension) ($P$ = 0.003), while no statistically significant differences were observed for both BMI and blood groups. The results of our current study showed that the frequency of Ct values between 16 and 26 was more frequent in ages older than 45 years, as well as in patients with severe to critical conditions and in those with diabetes compared to younger ages and those with mild to moderate conditions and patients with high blood pressure (30 vs. 20, 42 vs. 9, and 21 vs. 8), respectively [Table 3].

For the purpose of knowing the frequency of blood types in the group of mild-moderate COVID-19 patients, the group of patients with severe-critical conditions, and the group of recoveries, appropriate statistical calculations were performed, which clearly showed that there were no statistical differences ($P = 0.842$) between these three groups with regard to the frequencies of blood types [Table 4].

## Discussion

It seems clear from the results of the current study that older ages are more susceptible to contract COVID-19 ($P < 0.01$) [Table 1]. This is a realistic result due to the strength of immunity enjoyed by the younger ages, in addition to the significant role played by chronic diseases associated with old ages. Most studies indicate that age is the main player in the emergence of infection and its future repercussions,[11] and it may play a role in worsening COVID-19 predisposition.[12] A significant increase in the severity of the disease was observed in older age groups compared to younger groups.[13] Multiple reports have indicated that the majority of COVID-19 infections and severe cases occur in the elderly, but the mechanism underlying this is still unknown. Despite the fact that the severity of COVID-19 is associated with immune system dysfunction in older people, immunosenescence has recently been identified as a major factor in worsening COVID-19. Cytokine storm is also associated with COVID-19 severity and plays a very important role in the progression of COVID-19. Inflammation and immunological aging make it harder for older people to fight off SARS-CoV-2 infection.[14]

As for sex, although females recovered (58%) more than males (42%), this did not constitute a statistically significant difference, and this may be the reason behind some studies believing that the incidence of infection is equal between the sexes, but the cure rate is higher for females because their immunity is stronger than males. Studies have indicated that females exhibit higher humoral, antiviral, and inflammatory immune responses than males. Estrogen may be a useful ally in helping females with SARS-COV-2 illness.[15]

In addition, the role played by the sample size, time, and place of occurrence may be the reason behind this. These results are consistent in some aspects with some studies and differ with other studies in other aspects, as noted by Zhu *et al*.,[16] who showed a higher prevalence of COVID-19 infection in males compared to females, despite the fact that men vs. women had the same incidence and men with COVID-19 are more likely to die regardless of their age.

On the other hand, there are significant differences between the three groups under study with regard to BMI. It showed an increase in the incidence of COVID-19 disease for overweight/obese (67.8%) compared to normal weights (32.2%) in the patients group which is consistent with several previous studies showing an increased incidence and risk of COVID-19 disease in people who are overweight or obese.[17] Obesity is a risk factor that warns of the severity of the repercussions of this disease.[18] Severe obesity is behind the decline in lung function and thus the increased risk of COVID-19 disease.[19]

As for blood, no significant differences were observed regarding the different blood groups ($P = 0.099$). These findings do not match with Halim *et al*.,[20] who found that blood group A scored the highest frequency than the AB group in COVID-19 patients. Undoubtedly, these results indicate that ABO antigens play an important role in the pathogenesis of COVID-19. Therefore, antibodies to A and B which are normally produced, may affect susceptibility to COVID-19 infection.[21]

Regarding chronic diseases such as diabetes and high blood pressure, the percentage of those who did not suffer from these two diseases was significantly greater than the percentage of those suffering from them in the patient group. This clearly indicates that the absence of these two

**Table 3: Frequency of Ct value in COVID-19 patients stratified by age, disease severity, chronic diseases, BMI, and blood groups**

| Characters | | Ct value | | *P* value |
|---|---|---|---|---|
| | | 16–26 | 27–36 | |
| Age | ≤45 | 20 | 26 | 0.02 |
| | >45 | 30 | 14 | |
| Severity | Mild-moderate | 9 | 36 | <0.001 |
| | Severe-critical | 42 | 3 | |
| Chronic disease | DM | 21 | 10 | 0.003 |
| | HT | 8 | 20 | |
| BMI | NW | 20 | 9 | 0.081 |
| | OW | 31 | 30 | |
| Blood group | A | 17 | 10 | 0.749 |
| | B | 9 | 10 | |
| | O | 18 | 13 | |
| | AB | 8 | 5 | |

BMI: body mass index; DM: diabetes mellitus; HT: hypertension; NW: normal weight, OW: overweight/obese; *P*: the probability of Pearson's Chi-square test (to compare categorical variables)

**Table 4: Frequency of blood groups in COVID-19 mild-moderate patients group, severe-critical patients group, and recovered group**

| Blood groups | Mild-moderate ($n = 45$) | Severe-critical ($n = 45$) | Recovered ($n = 50$) |
|---|---|---|---|
| A | 17 | 11 | 15 |
| B | 9 | 10 | 12 |
| O | 14 | 18 | 15 |
| AB | 5 | 6 | 8 |
| Statistical analysis | Pearson $\chi^2 = 2.732$; DF = 6; $P = 0.842$ (NS) | | |

DF: degree of freedom; *P:* probability; NS: not significant ($P > 0.05$)

diseases does not prevent infection with COVID-19. It is evident from our study that the recovery rate of COVID-19 patients without these chronic diseases is higher compared to those who are affected by them.

The truth is that chronic diseases, whatever their type, must have a role in obstructing the recovery of patients infected with the COVID-19 virus, because of their role in obstructing the various defenses of the body from working smoothly, in addition to the damage they caused in various tissues and organs, which helps to exacerbate various other diseases. It is worth noting that most cases of cardiovascular disease, high blood pressure, diabetes, and respiratory disorders are associated with obesity and have a close relationship with the risk of infection with various diseases.[22] It was also reported that COVID-19 with high blood pressure delayed virus clearance and exacerbated excessive bronchitis.[23] On the other hand, it was observed that COVID-19 patients showed different levels of viral loads, as the relationship between viral load and severity of this disease is still unknown.[24]

Interestingly, this study showed that there is no clear role for COVID-19 in increasing or decreasing hemoglobin levels, as no significant difference was observed between the study groups, which gives the impression that this virus does not attack red blood cells. To date, researchers and physicians have recorded only a paucity of reports of patients with hemoglobin disorders in individuals infected with COVID-19.[25]

In complete contrast, significant statistical increases were observed between the study groups for each of the WBC counts, NLR, and concentrations of CRP, ferritin, D-dimer, and LDH. These inflammatory markers were significantly elevated in severe-critical cases compared to mild-moderate cases, while they returned to their normal levels in recovered groups, which definitively indicates the role of these inflammatory parameters in this disease and thus its severity and aggravation [Table 2].

Knowing changes in these predictive inflammatory and hematological markers can identify critically ill patients who require more aggressive medical intervention.[26] In fact, significantly elevated levels of key inflammatory markers including WBC, NLR, CRP, ferritin, D-dimer, LDH, as well as various inflammatory cytokines and chemokines, have been found to be unambiguously associated with more severe clinical conditions in patients with COVID-19.[27] Abdul Hussein and Fadhil[7] also indicated a significant increase in levels of these inflammatory markers in patients with severe-critical illness compared to patients with mild–moderate illness. Another study also indicated the role of age, CRP, NLR, and SARS-CoV-2 RT-PCR Ct in susceptibility to severity of COVID-19.[28] In addition, Ghazzi and Fadhil[29] pointed out that level of CRP during COVID-19 infection was effective biomarkers for the development of infection severity.

The current findings showed that COVID-19 patients over 45 years of age, as well as those with severe to critical disease and cases with a high viral load, showed significantly higher levels of CRP, ferritin, D-dimer, and LDH concentrations compared to younger patients and mild to moderate cases, and those with a lower viral load, respectively [Figure 1].

These results are not isolated from the results of other studies, as they have been consistent with those that indicated that CRP levels in the blood are a critical marker of the severity and progression of COVID-19, which is also associated with a higher viral load and age among COVID-19 patients, and this level can help distinguish between individuals with moderate to severe COVID-19 infection.[30] Furthermore, it has been reported that severe disease was associated with higher Ct values and with advanced age.[31]

In a related context, serum ferritin, as an "acute phase reactant," reflects the degree of chronic as well as acute inflammatory reaction within the body, which is associated with age, disease severity, and increased viral load.[32] On the other hand, several studies have linked a high level of D-dimer with disease severity and increased viral load, especially at older ages, which will increase the risks and adverse outcomes of COVID-19.[33] Also, studies to date have shown that patients with severe cases of COVID-19 have significantly elevated levels of LDH in their blood and a sixfold increased risk of severe COVID-19 in these patients.[34]

The findings of the current study showed that the frequency of Ct values between 16 and 26 was more frequent in ages older than 45 years, as well as in patients suffering from severe-critical conditions and in patients with diabetes compared to younger ages, those with mild-moderate conditions, and patients with high blood pressure, respectively [Table 3].

This seems to be a logical and realistic result, as the risk of the disease increases in older ages due to the weakness of their immune systems, what if this is accompanied by their suffering from chronic diseases such as diabetes? Therefore, an increase in Ct values in these groups seems to be an inevitable consequence.

The severity of COVID-19 disease is closely related to higher viral load and aging, especially for those with chronic diseases. Several previous studies have confirmed that patients with diabetes mellitus are at risk of developing severe infection with coronavirus (COVID-19), compared to patients without diabetes. Furthermore, these patients are believed to be at higher risk of later complications and risk of death due to these comorbidities.[35]

On the other hand, recent studies have looked at blood groups as a risk factor for COVID-19 disease.[36] Blood group antigens play a direct role in infection through

various mechanisms. At the molecular level, they can act as receptors for pathogens. It can also facilitate the intracellular uptake of viral particles.[37,38]

The researchers found that individuals with the type O blood group were less likely to be infected with SARS-CoV-2 than those with other blood types. Interestingly, individuals with an O-negative blood group were more protected against viral infection. Type O individuals were at lower risk compared to nontype O individuals for secondary outcomes, such as severe illness and death. Rh-negative individuals also had a lower risk of severe disease and mortality compared to Rh-positive patients. Together, the researchers concluded that O and Rh-negative blood types may be protective against SARS-CoV-2 infection, whereas individuals with blood type A were at greater risk of infection than individuals with blood type O.[39]

With regard to antibodies, anti-A antibody was observed to block the interaction between SARS-CoV-1 and the angiotensin-converting enzyme 2 receptor, which indicates that ABO antibodies may affect SARS-CoV-2 infection as well. Although this appears to be an emerging trend, the effect of blood type on clinical outcomes remains unclear.[40-42]

Although the results of our current study do not support that blood type is part of a predictive model for COVID-19 disease [Table 4] and is not related to the severity of infection or recovery from it, therefore ABO/Rh testing should not be used as a screening mechanism for this purpose. However, future investigations could focus on creating a global COVID-19 database to account for population differences in blood types and testing protocols. In addition, more studies are needed to understand the molecular mechanisms by which blood types may generate susceptibility to SARS-CoV-2 infection and, ultimately, to develop countermeasures for viral infections and diseases.

## Conclusion

Old age and being overweight can be considered risk factors for contracting the COVID-19 disease, and healthy people who do not suffer from chronic diseases or young people are not immune from contracting this disease. The markers of inflammation (WBCs, NLR, CRP, ferritin, D-dimer, and LDH) can serve as important indicators for predicting the severity of COVID-19 disease. Lower Ct values have been associated with patients at older ages, especially those with severe infections and chronic diseases such as diabetes that correlate positively with higher concentrations of CRP, D-dimer, LDH, and ferritin.

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### Conflict of interest

There are no conflicts of interest.

### Ethical approval and institutional review board statement

The research was conducted adhering to ethical principles that were first established by the Helsinki Declaration. Each participant completed a written consent form before blood and NP swabs were taken. The permission form, subject information, and study protocol were reviewed and approved by the Mustansiriyah University/College of Science/Ethics Committee on September 1, 2022. Under reference number BCSMU/1221/00012M, the approval was given.

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