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Determination of Risk Factors and Some Biomarkers Parameters during Infected Iraqis with Covid-19

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Abstract

Covid-19 is a global challenge that drives health care to its limits. Biological parameters associated with increased risk of severe or fatal disease courses were identified, including conditions connected with a poor state of health, organ damage, and coagulation dysfunction. In a recent study, risk factors like age, sex, blood group (ABO), smoking, and several biomarkers like white blood cells, lymphocytes, C-reactive protein (CRP), ferritin, D-dimer, and potassium level were considered. One hundred nasopharyngeal swabs and blood samples were collected from Iraqi patients and classified according to the severity of the disease into five groups: Asymptomatic, Mild, Moderate, Severe, and Deceased, according to the recommendations of the World Health Organization (WHO). The current study was based on the risk factors and their effect on disease severity (age, sex, blood type, and smoking). As a result, there was a substantial positive linear link (R2=0.91) between getting older and increasing disease severity. Study results were showed a little statistically significant difference in the number of males and females infected with SARS-CoV-2. Interestingly, males were shown to be more susceptible to infection than females. Also, because type (O) blood is more extensively distributed in the Middle East population than the other blood types, those with type (O) blood are more susceptible to infection. Because smokers and non-smokers were distributed at random across different disease severity levels, there was no statistically significant link between smoking status and COVID-19 severity. All parameters (plasma protein and liver enzymes) showed statistically significant differences, especially between the deceased groups except K level, which did not differ significantly in all groups. This study aimed to investigate some biological markers during infection to see if there was a link between these measurements and risk factors, as well as the severity and progression of the disease.

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1. Introduction

A new respiratory disease, the coronavirus disease 2019 (Covid-19), emerged at the end of 2019. Next-generation sequencing identified the disease-causing pathogen as a novel coronavirus closely linked to the SARS-corona virus discovered in 2003[1]. Over the last two decades, coronaviruses have been blamed for a slew of health issues. The COVID-19 viruses, according to WHO data, had a higher overall death rate (around 3.3 percent) than previous influenza pandemics in 1918 and 1957, and their spread rate was 40 times greater [2]. Fever, cough, myalgia, and fatigue are common symptoms of Covid-19, while headache, sputum formation, hematuria, and diarrhea are less common. During the illness, a small percentage of patients develop pneumonia with irregular chest CT findings [3]. The virus is often detected in throat swabs using real-time quantitative polymerase chain reaction (rt-PCR) [4, 5]. The elderly has a higher mortality rate. In Korea and Italy, approximately 80% and 90% of deaths occurred in patients over the age of 70 and 60% in patients under the age of 60, respectively. In other COVID-19 affected countries, a similar trend was observed. According to many studies, old age is a major risk factor for COVID-19 mortality [6, 7]. The time between admission to the hospital and death, as well as viral clearance, is influenced by age [8, 9]. Many studies from China, Spain, and Italy found that the proportion of men who died as a result of the infection was much higher than that of women, which may be due to gender differences in tobacco use, physical activity, dietary habits, occupational exposure to smoke and dust, and other co-morbidities [10, 11]. Several findings during the serious acute respiratory syndrome coronavirus (SARS-CoV-2) outbreak indicated that ABO type may play a role in the disease, with group O individuals being less susceptible. In most trials, COVID19 patients had a higher proportion of group A and a lower proportion of group O than safe controls. Another study found that group AB patients had a higher infection rate than group O patients. In comparison, another study found no connection between group A status and COVID19; however, group O people had a lower risk of COVID19, while group B and AB people had a higher risk [12, 13]. The relationship between the Rhesus blood group (e.g. Rh (D) type) and COVID19 has also been studied. According to one report, Rh (D) positive people were more likely to test positive for SARS-CoV-2. Another study discovered significant links between Rh (D) blood group status, group B, and SARS-CoV-2 infection. Several observational studies have recently revealed an inverse relationship between smoking, coronavirus disease 2019 (COVID-19), infection mechanisms, and disease spread. There is no conclusive evidence that smokers are immune to the SARS-CoV-2 virus [14]. The ratio of increased white blood cell counts to depleted lymphocyte counts has been indicated as a prognostic biomarker in COVID-19 patients [15, 16]. CRP levels may indicate the seriousness of a disease and should be used as a key indicator for disease surveillance. CRP levels can activate complement and increase phagocytosis, clearing the body of pathogenic microorganisms. CRP levels can be used to detect pneumonia early on [17, 18]. Ferritin is a key mediator of immune dysregulation, particularly in severe hyperferritinemia, contributing to the cytokine storm through direct immune-suppressive and pro-inflammatory effects. It has been recorded that COVID-19-related fatalities are followed by cytokine storm syndrome implying that disease severity is influenced by cytokine storm syndrome [19-23]. A D-dimer is a Fibrin Degradation Product (or FDP), a small protein fragment discovered inside the blood after fibrinolysis breaks down a blood clot. D-dimer assays are frequently utilized in medical exercise to rule out a prognosis of deep vein thrombosis or pulmonary embolism, and an excessive D-dimer shows a greater threat of peculiar blood clotting. Increased D-dimer ranges have additionally been related to a better mortality rate in community-received pneumonia [24-26]. Worldwide, thrombotic diseases are the leading cause of death. The creation of intravascular thrombus, which leads to death, was induced by a variety of cardiovascular illnesses (CVDs). According to the American Heart Association's 2011 data, thrombosis was responsible for 31.3 percent of deaths, while the WHO reported that 17 million people died each year from thrombotic illnesses [27]. Nano-materials, on the other hand, can be a beneficial tool for reducing the negative effects of radiation in virtually normal tumor cells, and nano-particles with large atomic numbers can be successful as antiviral disinfectants in the field of surface sterilization. Self-sterilizing surfaces based on nanotechnology and their potential to combat COVID-19 [28]. This study aimed to investigate some biological markers during infection to see if there was a link between these measurements and risk factors, as well as the severity and progression of the disease.

2. Materials and Methods

2.1. Study Design

A study was conducted after the approval of the ethical committee at the College of Biotechnology-University of Technology. One hundred nasopharyngeal swaps (Sigma Virocult Company, UK) were collected with viral transport medium according to Bradford and Slavin (1940), and stored frozen at-70°C until used for RNA

extraction and polymerase chain reaction in addition to the venous blood samples that were distributed in serum gel tubes and EDTA tubes for serum and plasma preparation respectively. Patients were classified as asymptomatic, mild, moderate, severe, and critical cases from October 2020 to February 2021 at Al-Kindy Hospital and Central Public Health Laboratories (CPHL). A specially designed questionnaire was used for patient case information detection.

2.2. Reveres Real Time PCR Detection

Viral RNA was extracted by the Qiamp viral RNA extraction kit from (Qiagene, Germany) according to the manufacturing instructions. The extracted RNA was preserved at -70 till use. Qualitative multiplex RT-rPCR detection of the E gene, RdRp, N gene, and internal positive control using the SARS-CoV-2 Multiplex Real-Time RT-PCR Kit (Bioneer/Korea). The following steps are included in the PCR running program as is shown in Table 1.

Table 1: The PCR Run program protocol.

Steps	Temperature (◦C)	Time	Cycle
Reverse transcription	50	20 min	1
Pre-denaturation	95	5 min	1
Touchdown	95	5 Sec.	5
	60	30 Sec.	40
Denaturation	95	5 Sec.	
Annealing & extension	58	30 Sec.	
	Holding 4^{o}C		

2.3. Blood Group Test and White B Cells and Lymphocyte Counting

Fresh whole blood samples were used for blood group testing. The tests were done for all blood samples collected from patients using the Monoclonal IgM Antibodies kit provided by (Cam Tech Medical kit/UK), based on the agglutination principle, where red cells processing the antigen agglutinate in the presence of the corresponding antibody, indicating that the result is positive. Fresh whole blood samples were used for WBC, and Lymphocyte counting using the D-Cell 60 DIGON ltd (Europe/Hungary) as a fully automated haematology analyzer device.

2.4. Biochemical Parameters (CRP, D-dimer, Ferritin, and Potassium level) Determination

Serum was separated from blood collected in a serum gel tube to determine the CRP, and potassium levels in the patient's serum using (Roche Diagnostic cobasC311/Germany). Ferritin was detected by using (Roche Diagnostic Cobas e411/Germany). The prepared plasma was used to determine the D-Dimer levels using (Roche Diagnostic Cobas C311).

3. Results and Discussion

3.1. Distribution of Risk Factors

The outcomes existing in this study were based on one hundred nasopharyngeal swaps and venous blood samples. One hundred patients were distributed over five groups with different frequencies. The spectrum of illness severity among patients infected with COVID 19 can be divided according to the severity of illness depending on WHO guidelines. Among the 100 patients, 50 patients were hospitalized in Al-Kindy in the isolation hall and intensive care unit, as shown in Figure 1.

3.1.1. Age Distribution

The study showed that the relation between age and disease severity had been investigated. The patients' age that was asymptomatic ranged from 19 to 59 years (15%), mild patients ranged from 16 to 74 years (22%), moderate patients ranged from 30 to 76 years (13%), and severe patients ranged from 40 to 90 years (41%). The mean age of all patients in this study was 52.4 years, as shown in Figure 2. The age distribution showed a statistically significant difference among different disease severities with a strong positive linear relationship (R2=0.91) between increasing age and increasing disease severity as shown in Figure 3. In Iraqi patients, age is a significant risk factor for COVID-19. The average patient age was approaching the fifth decade (48.2±13.8 years), and 48.3 % of patients were under the age of 50. These findings are strongly compatible with our finding the severity and

outcome of the disease are heavily influenced by the patient's age showing that those over the age of 59.8±10.5 are more likely to develop severe COVID-19. According to Mueller, and others [1, 29, 30] the study refers to that COVID-19 is more common in the elderly than in younger people. Furthermore, the majority of hospitalized COVID-19 cases (80%) were 65 years or older, and they had a 23-fold higher risk of death than younger patients. COVID-19 has been revealed to be particularly deadly to the elderly, according to Williamson and colleagues [31]: in the United Kingdom, 90% of COVID-related deaths occurred in those over 60, and people aged 80 and up were found to be at a 20-fold higher risk than those aged 50 to 59.

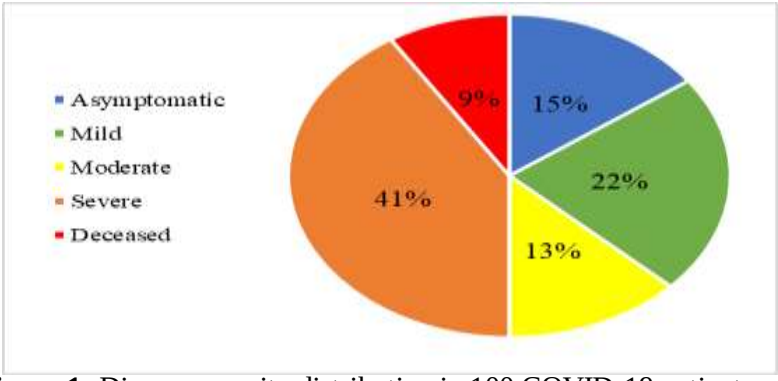

Figure 1: Disease severity distribution in 100 COVID-19 patients.

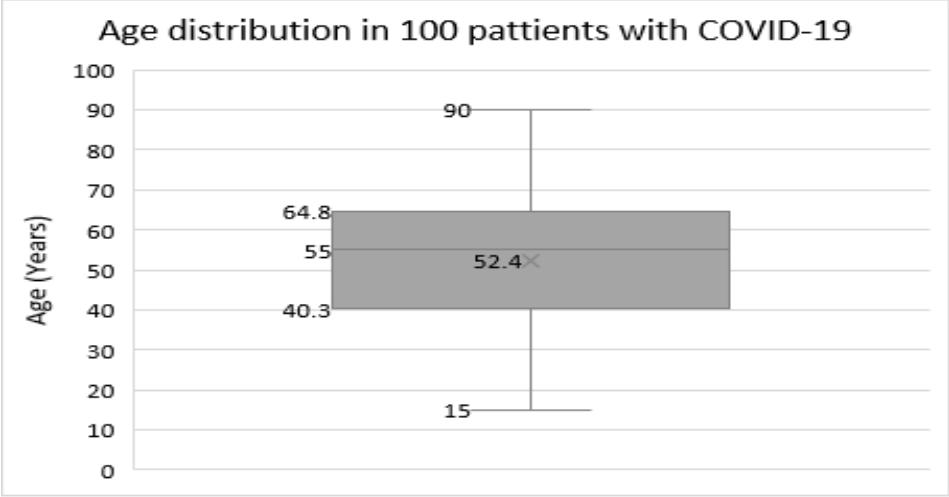

Figure 2: Distribution of age in 100 patients with COVID-19.

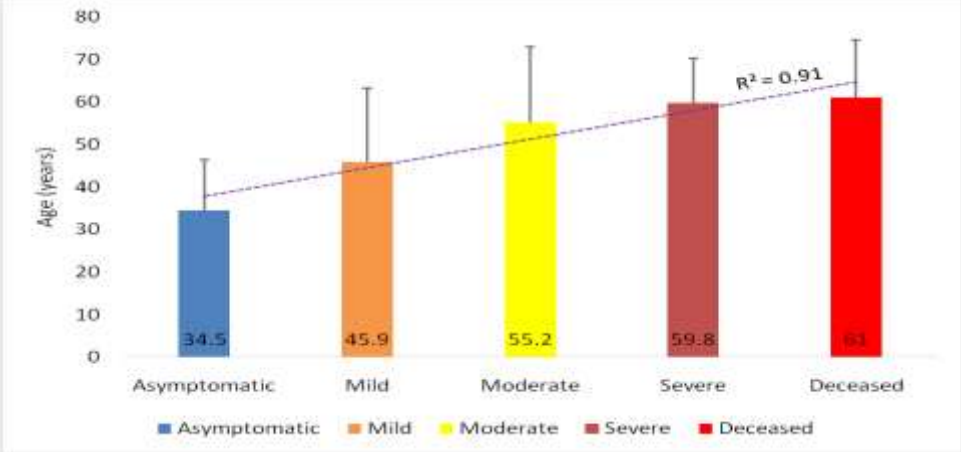

Figure 3: The difference in age (years) in 100 individuals with COVID-19 of various severity levels.

3.1.2. Sex Distribution

The results revealed that 38% were female and 62% male, respectively as shown in Figure 4.

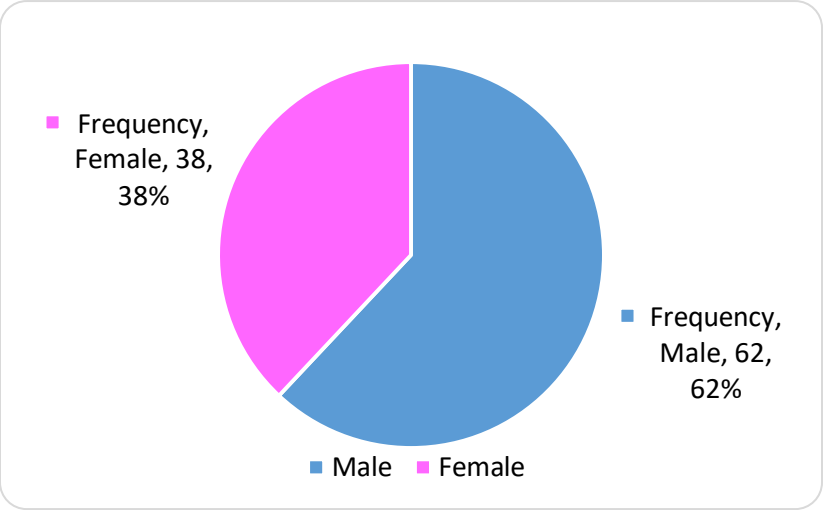

Figure 4: Sex distribution in 100 patients with COVID-19.

The sex difference in COVID-19 illness severity could be due to gender-based socio-cultural and behavioral variations. Men are less likely to wash their hands with soap after using the restroom [32], and men are more likely to leave the house and go to crowded places in Iraqi cultures. Infection rates may be skewed towards a male bias due to unequal access to healthcare and testing. Among the important reasons that explain the infection of males more than females are Sharma and others. [33], refers to that the men and women have different innate and adaptive immune responses, which may be linked to X-chromosomal-inherited sex-specific inflammatory responses. Since the X chromosome has a high density of immune-related genes, women's innate and adaptive immune responses are usually greater than men's. Sex chromosome genes and sex hormones, such as estrogen, progesterone, and androgens, may play a role in the differential control of immune responses in men and women. The existence of disease susceptibility genes, sex-dependent development of steroid hormones, and different copy numbers of immune response X-linked genes are all factors that contribute to sex-specific disease outcomes after viral infections. Early data from China found that hospitalized patients were mostly men, with a median age of 56 years; 26% required intensive care unit (ICU) care, and 28% died [33].

3.1.3. Blood Typing Distribution

The most common blood group encountered was O+ followed by A+, B+, and AB+ respectively, and the least encountered groups were A- & B-> None of the collected cases were O- or AB- blood groups, as shown in the pie chart of Figure 5.

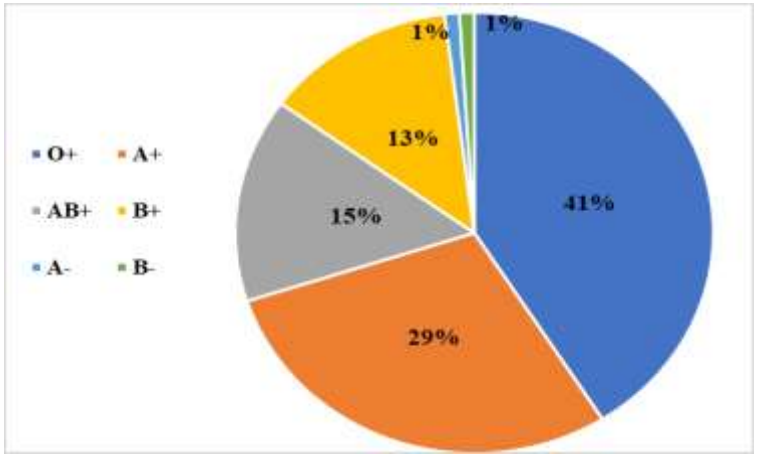

Figure 5: Blood groups distribution in 100 patients with COVID-19.

Polymorphisms in the ABO gene are reflected in the ABO blood type trait. This gene has been linked to several other features, including COVID-19 morbidity and mortality risk factors. Variants within ABO, for example, have been linked to angiotensin-converting enzyme activity in genome-wide association studies [34]. Fan and others, [35] in a Chinese study (Wuhan city), pointed out that the frequency of group (A) was considerably higher in COVID-19 patients compared to controls, but the frequencies of groups (B, AB, and O) were not significantly different. According to a Spanish study, group O patients had a lesser vulnerability to COVID-19, while group B patients have a higher chance of problems [36]. Susceptibility to COVID-19 was associated with a group (AB) in patients from Baghdad in a previous Iraqi study undertaken by our group, whereas group A was associated with an increased risk of death [37]. Another retrospective research found similar results but did not go into detail about the co-morbidities. Another research found that group (AB) patients had a higher infection rate than group (O) patients, in comparison, another study found no connection between group (A) status and COVID19; however, group O people had a lower risk of COVID19, while group (B) and (AB) people had a higher risk [35, 38-40]. Our study could be linked to prior research that found that in both males and females, the general trend for the ABO blood group was (O > A > B > AB), with blood group (O) having the most pronounced phenotypic and blood group AB being the least distinguishable among the population studied [14, 41-43]. Studies indicate that the Iraqi society and most of the Middle East countries indicate that the results of the most prevalent blood type were (O) (37.16%), followed by blood type A (32.47%) and then B (23.84%), while the least prevalent blood type was AB (6.53%). The majority, 91.73%, was Rh-positive, and 8.27% were Rh-negative [44]. These findings support our research, which found that people with type (O) blood are more susceptible to infection because their population spreads more widely than the other blood types.

3.1.4. Smoking Distribution

Twenty-nine of the selected cases were smokers (28 males & 1 female). Smokers & nonsmokers were randomly distributed among different disease severities and thus, there was a statistically non-significant association between smoking status & COVID-19 severity as shown in Table 2. Tobacco use has been linked to a variety of respiratory disorders, and significant data has shown that it has a deleterious impact on lung health [45]. Smoking impairs the immune system's ability to respond to infections, rendering smokers more susceptible to infectious diseases [46]. Arcavi and others, [15, 47] found during the previous MERS-CoV epidemic, smokers were twice as likely as nonsmokers to get influenza and experience more severe symptoms, as well as having a higher mortality rate not in COVID-19.

Table 2: Distribution of smokers in 100 patients with COVID-19.

Disease severity	Smoking status		P-value
	Non-smoker	Smoker	
Asymptomatic	14.1%	17.2%	0.193
Mild	19.7%	27.6%	
Moderate	15.5%	6.9%	
Severe	38%	48.3%	
Deceased	12.7%	0%	

3.2. Differences between White Blood Cells (WBC) & Lymphocyte Counts

White blood cell counts showed irregular fluctuations among (polynomial relation with) different disease severities. They were highest in “moderate” cases and lowest in “mild” cases. The overall difference was not statistically significant. Lymphocyte counts; on the other hand; showed a statistically significant inverse linear correlation with disease severity. The counts decreased progressively from “asymptomatic” & “mild” cases to more severe forms. The decrease was most significant in “severe” cases and to a lesser extent in “moderate” cases as is shown in Table 3, and Figure 6. White blood cell counts, on the other hand, changed fluctuated irregularly throughout disease severity levels (polynomial relationship). In moderate situations, they were the highest, while in mild cases, they were the lowest. The difference was not statistically significant in the aggregate. Olga Pozdnyakova and others [29], found that COVID-19 patients had significant quantitative and morphologic changes in their WBCs, which differed between moderate and severe illness. Significant and lymphopenia were related to more severe illness, which was exacerbated in critically ill patients. However, an abnormal WBC count,

particularly in lymphocytes, was linked to milder disease. As a result, in the early stages of the disease, the number of WBCs drops while the number of lymphocytes rises. All WBCs revert to pre-infection levels after the end of the infection, although the number of lymphocytes reduces dramatically, which is consistent with our findings when samples are taken from hospital patients. Their results indicate that they are in the middle of an infection, whereas the rest of the groups' results indicate that symptoms have begun to appear. On the other hand, lymphocyte numbers indicated a statistically significant inverse linear relationship with illness severity, Lymphopenia is a prevalent biomarker in SARS-COV-2 infected patients, and it indicates a faulty immunological response to the illness, as is shown in Figure 6.

Table 3: White blood cell (WBC) and lymphocyte counts ($x10^9$/L) in 100 patients with COVID-19 revealed different severities.

Disease severity	WBC count	Lymphocyte count
(a) Asymptomatic	6.5±3	3.4±1.4
(b) Mild	5.1±2	3.5±0.9
(c) Moderate	7.4±3.3	2.1±1.6 [(b)]
(d) Severe	6.9±3.6	2.2±1.5 [(a,b)]
(e) Deceased	7.1±3.9	2.4±1.6
P-value	0.219	0.001

*(Data presented as means±SD, superscript letters represent the statistical significance with groups of the same letter, P-value<0.05). * a=Asymptomatic, b=Mild, c=Moderate, d=Sever, e=Deceased.

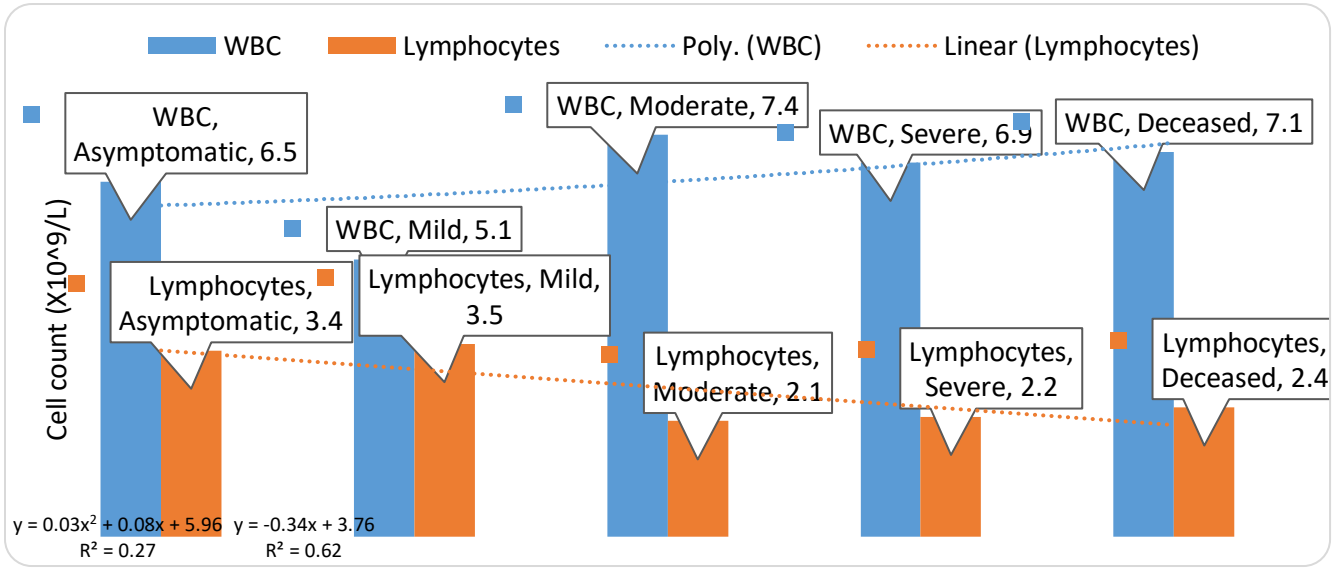

Figure 6: WBC & lymphocyte counts among 100 patients with COVID-19 of different severities.

3.3. Differences in Proteins (CRP), D-dimer, and Serum Ferritin

These three parameters showed statistically significant differences, especially between the deceased group on one hand and the asymptomatic and mild groups on the other.

3.3.1. CRP Levels

CRP levels showed a strong exponential relation (R2=0.98) with disease severity. Increased severity was associated with a progressive increase in CRP levels as is shown in Figure 7. CRP Increases levels in this study could suggest a high level of inflammatory stress, which could lead to serious/critical sickness or death. In COVID-19 infected people, a link between CRP concentration and lung lesion has been discovered; however, Koozi and others, [48-51] were showed that elevated CRP levels are associated with a 30-day mortality rate; furthermore, it was discovered that the level of CRP can be used to track the progression and improvement of COVID-19 patients. Despite its usefulness in predicting a poor prognosis, CRP levels can be affected by a variety of factors, including liver injury, blood pressure, lipid levels, weight, smoking status, gender, and age.

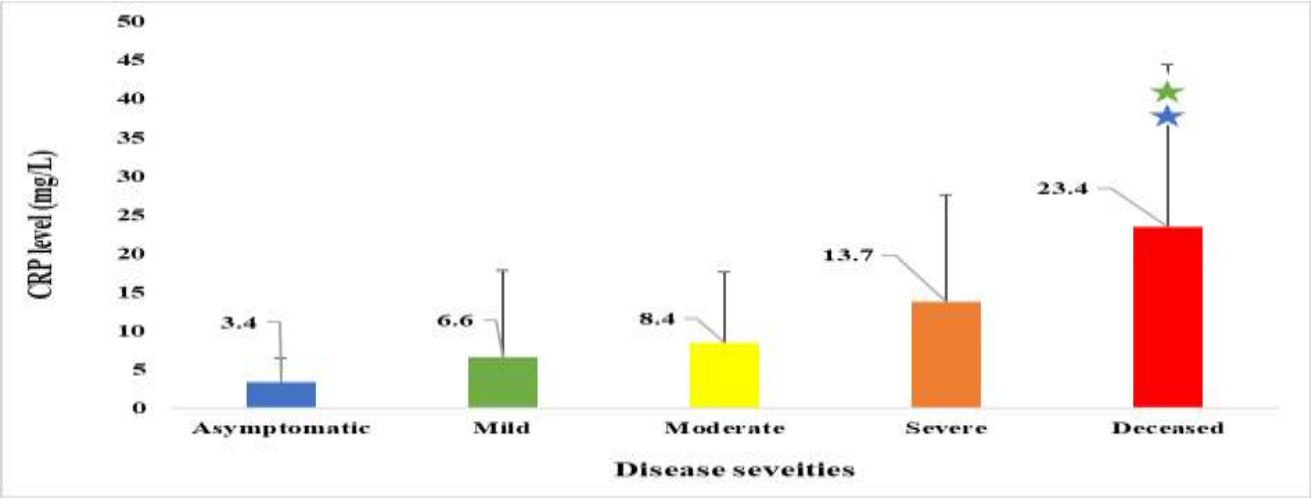

Figure 7: C-reactive protein (CRP) levels in 100 patients with COVID-19 of different severities.

3.3.2. D-dimer Levels

D-dimer was significantly increased in deceased cases in comparison to the low levels in mild and moderate cases. It showed strong polynomial regression (R2=0.95) with disease severity, first decreasing from asymptomatic to its lowest level in moderate cases before increasing again to its heist levels in "deceased" cases as is shown in Figure 8. COVID-19 disease development may be linked to higher D-dimer levels. Huang and others, [52, 53] found that the amount of D-dimer was found to be considerably higher in COVID-19 patients admitted to the ICU, patients with severe COVID-19, who were often bedridden and had impaired coagulation function, should be given special attention in terms of venous thromboembolism risk. Inflammatory cytokines may produce an imbalance of coagulation and fibrinolysis in the alveoli, which may activate the fibrinolysis system, resulting in an increase in D-dimer levels [54, 55].

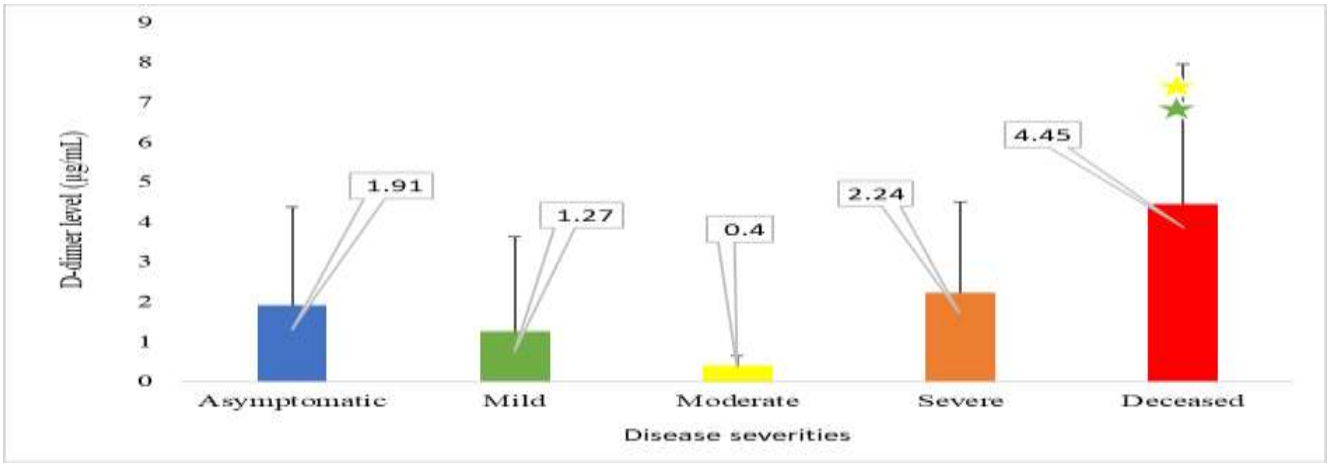

Figure 8: D-dimer levels in 100 patients with COVID-19 of different groups.

3.3.3. Ferritin Level

Serum ferritin levels showed strong polynomial relation (R2=0.79), with fluctuating levels in different disease severities. Statistically significant high levels were seen in moderate, severe and deceased cases, as is shown in Figure 9. Serum ferritin is a well-known inflammatory marker that rises in response to systemic and pulmonary inflammation, as well as several disorders including COVID-19 [56]. Ruddell and others, [57] found the mechanisms underlying the link between elevated ferritin levels and infection severity in COVID-19 patients are unknown; however, pro-inflammatory cytokines that may promote ferritin production are one possibility. Cellular damage is caused by an inflammatory process that enhances intracellular ferritin leakage [58].

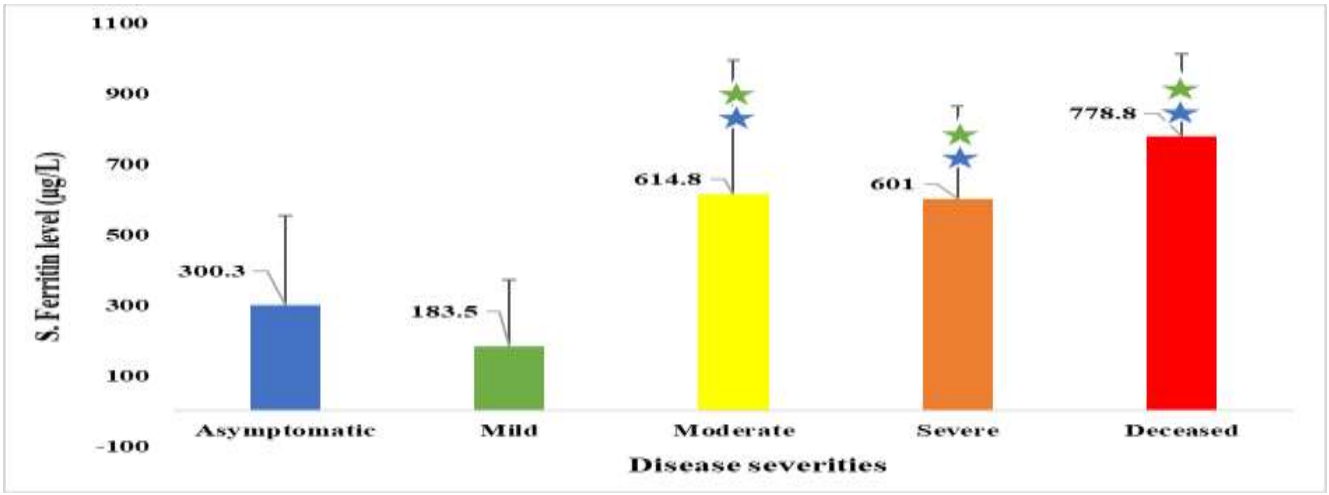

Figure 9: Ferritin levels in 100 patients with COVID-19 of different severities.

3.4. Differences in Serum Potassium Levels

Serum potassium levels did not differ significantly in any of the groups, although they did decrease slightly but not significantly in asymptomatic patients for a variety of reasons, including sampling conditions, timing, patient health history, and treatment, as is shown in Figure 10. Tongyoo and colleagues, [59] have found a relationship between potassium levels and disease prognosis; both hypokalemia and hyperkalemia have a poor prognostic value. Patients in the medical ICU with abnormal K+ levels had a greater rate of ICU death than patients with normal K+ levels, according to prospective cohort research. Increased serum potassium was found to be an independent predictor of mortality in individuals with severe community-acquired pneumonia in a prospective observational study [60]. As a result, understanding how different indicators behave after a disease has progressed could aid in recognizing the severe disease and, improving prognosis. In this investigation, serum potassium levels were not statistically different in any of the categories; however, they were marginally but not significantly lower in asymptomatic cases as is shown in Figure 10.

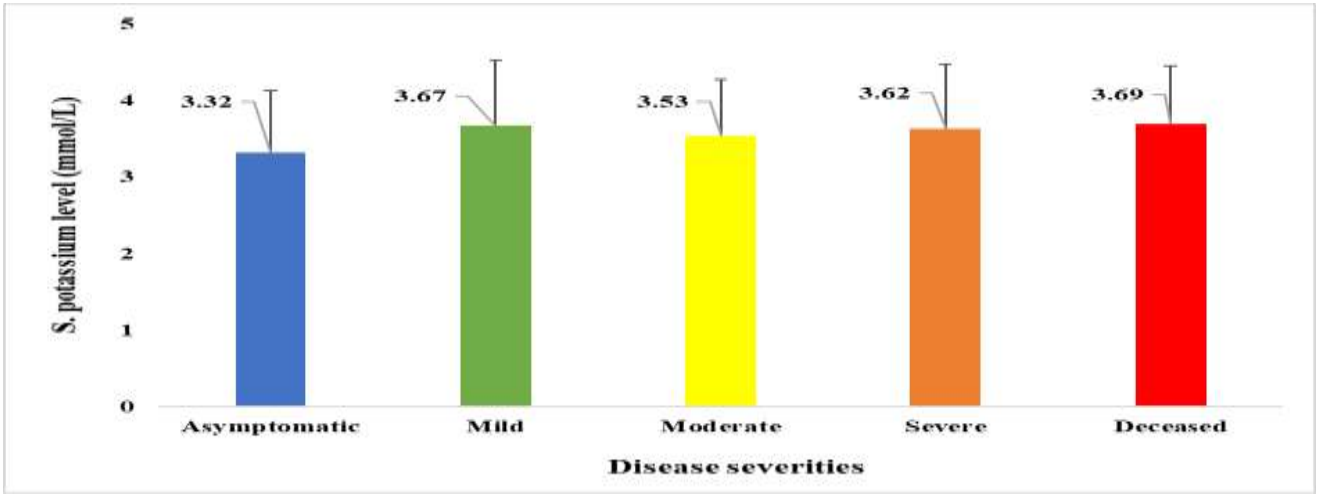

Figure 10: Potassium levels in 100 patients with COVID-19 revealed in different disease severities.

4. Conclusions

The results obtained from the present study revealed a substantial positive linear link between getting older and increasing disease severity, while there is a little statistically significant difference in the number of males and females infected with SARS-CoV-2. Interestingly, males were shown to be more susceptible to infection than females. On the other hand, blood groups of type (O) are more susceptible to infection, while blood groups of type (B) are more resistant among patients. Furthermore, smokers and non-smokers were distributed at random across

different disease severity levels, and there was no statistically significant link between smoking status and COVID-19 severity. Although white blood cell counts fluctuated irregularly throughout illness severity levels, lymphocyte counts indicated a statistically significant difference across groups of different disease severity levels. All parameters of plasma proteins showed statistically significant differences, especially between the deceased groups, except K level, which did not differ significantly in all groups.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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