Archives of Razi Institute, Vol. 78, No. 2 (2023) 737-742

DOI: 10.22092/ARI.2022.359731.2466

Original Article

Role of Serum Ferritin, D-Dimer, and C-Reactive Protein Parameters in COVID 19 Severity

Abdullateef Abdullah, Z[1], Fouad Ali, L[1]*

1. Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Received 16 August 2022; Accepted 7 September 2022
Corresponding Author: laylafouad1971@yahoo.com

Abstract

Following the epidemics caused by the transmission of the common virus between humans and animals (COVID-19), coronavirus 2 (SARS-CoV-2) is the third and most deadly strain of RNA virus that can cause respiratory, digestive, and nervous system problems, and there are many unknown complications. This study included 170 clinical samples of nasopharyngeal swaps (100 patients and 70 controls for both males and females). RT-PCR was performed, and blood samples were taken for biochemical analyses. They were obtained from Iraqi patients aged 25 to 92 years old. Between November 2021 and March 2022, COVID-19 patients were admitted to Dar al-salam Hospital, Alyarmok Teaching Hospital, and Alshefaa Hospital. AFIAS D-Dimer, AFIAS ferritin, and NycoCard CRP tests were performed on the patients and were classified depending on the severity of their infection (mild or moderate, severe and critical). The results showed a significant increase in ferritin in critically ill patients (545.58 ± 57.71). A significant increase of D-dimer was found with different severity with highly significant in the critical group (3.93 ± 0.79). With varying degrees of severity, a substantial rise in CRP was discovered with highly significant in the critical group (96.27 ± 14.55) between the severity group (*p*-value <0.001). Also, COVID-19 individuals in the age range (50 – 60) tended to be more severe than younger people, whereas the effect of gender is not significant in any patient group. The biochemical factors, including D-Dimer, ferritin, and CRP, are effective in the disease's occurrence of symptoms and severity.

Keywords: Biochemical parameters, COVID-19, RT-PCR, SARS-CoV-2, Serum ferritin, D-dimer, C-reactive protein

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by coronavirus 2 (SARS-CoV-2), which causes acute respiratory syndrome (ARDS), which first appeared in Wuhan, China, in December 2019 and has since expanded to the world (1, 2). This 2019-nCoV is the third and most dangerous human pathogen following a zoonotic transmission epidemic of CoV, SARS-CoV (in 2003), and MERS-CoV (in 2012); positive sense RNA was found which affects birds as well as a variety of other creatures including humans (3). Viral pneumonia and host inflammation characterize each of these diseases; as a result, pulmonary edema develops and a state resembling (ARDS) acute respiratory distress syndrome (4).

Coronaviruses are members of the Coronavirinae subfamily of the Coronaviridae family in the order Nidovirales. In humans and many other animals, it can induce respiratory, digestive, and neurological system disorders. The Coronavirus particles are spherical, varying from 80 to 160 mm. The spike (S) protein is coated on the envelope's surface. The S proteins are membrane (M) and envelope (E) proteins. A spiral nucleocapsid is made up of genomic RNA and phosphorylated nucleocapsid (N) protein and can be found inside the envelope (5). COVID-19 is a

pleomorphic or spherical encased particle; single-stranded (positive-sense) RNA is associated with a nucleoprotein within a matrix protein-based capsid. The envelope is adorned with club-shaped glycoprotein projections. Several coronaviruses have the hem agglutinin-esterase protein (HE) (6). Coronaviruses are RNA viruses with giant genomes (26.4–31.7 kb) with levels of G+ C ranging (from 32 – 43) %. A variety of small ORFs (spike, membrane, envelope, nucleocapsid, and ORF1ab) reside downstream of the nucleocapsid gene between many conserved genes and in different coronavirus lineages.

The spike protein's unique N-terminal region is one of the viral genome's defining features. S, E, M, and N are the genes that code for the essential structural proteins in all coronaviruses, and they exist in the 5′–3′ order (7). COVID-19 patients might present with a variety of symptoms. Given that those general findings, such as neurological and skin symptoms, show a range of individual reactions, the need to emphasize a large number of presentations cannot be overemphasized. This might lead to a rise in the number of symptoms and signs. In general, dyspnea, fever, cough, and headache are nonspecific common SARS-CoV 2 symptoms. From asymptomatic patients to those dying of severe pneumonia. The infection's severity may differ (8) shortness of breath, fever, and cough were the initial symptoms of the condition. The list was expanded to include chills, headache, muscle discomfort, sore throat, and loss of taste or smell (neurological manifestations) by the US Centers for Disease Control and Prevention (CDC). There are three severity degrees in the 2019 Coronavirus Disease levels. Flu, for example, may occur in the first stage due to viral pneumonia and infection. Patients may be admitted to the hospital or placed on a ventilator for a lengthy period. The second stage also distinguishes between pulmonary inflammation and coagulopathy, which can happen sequentially but overlap. The disease's last stage is lung fibrosis.

In individuals who require mechanical ventilation, two respiratory phenotypes can be distinguished low and high elastance. The H-type has more lung edema, resulting in a more considerable pulmonary weight and worse lung compliance (9). Due to the respiratory system's prominent involvement in suspected COVID-19 cases, a chest CT scan is strongly advised for initial screening and follow-up. X-rays of the chest have a limited diagnostic value in the early stages, even though CT scans can be obtained prior to the beginning of symptoms. Furthermore, When an initial false-negative result utilizing real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was obtained, CT results were diagnostic in a few cases (10). One of the critical challenges for facilitating public health initiatives is the reliability of biomarker testing. Real-time PCR is commonly utilized to identify causal viruses in respiratory secretions in acute respiratory infections (11). In a pandemic, laboratory biomarkers that predict the severity of COVID-19 are critical. The study aimed to investigate the relationship between some biomarkers using a systematic review and meta-analysis, including serum ferritin, D-dimer, and C-reactive protein, with the severity of COVID-19.

2. Materials and Methods

2.1. Samples Collection

This research was conducted on 100 males and females between the ages of 25 and 92years and 70 people as a controls group in Dar al-salam Hospital, Alyarmok Teaching Hospital, and Alshefaa Hospital (November 2021 to January 2022). The study included (100) Iraqi patients who had positive SARS-CoV nasopharyngeal swaps analyzed by real-time reverse transcriptase PCR and were hospitalized in different isolation wards. Those with vomiting, diarrhea, weakness, systemic disorders, and low oxygen saturation were taken to the hospital. On the hospital's second day of admission, blood specimens were collected by drawing venous blood from each patient and placing 3 ml of whole blood in three tubes. To test c-reactive protein, start with a non-heparinized tube (gel tube) (Nyco Card kit from Abbott USA company). The serum is removed and purified from it by

centrifuging it for 10 minutes at 3000 RPM (rpm). The D-dimer (Cardiac AFIAS D-Dimer kit from united medical company, China), the second tube contains sodium citrate (anticoagulant). The third tube is to test ferritin using plasma (boditech AFIAS Ferritin kit from united medical company, China).

2.2. Real-Time PCR Detection of SARS-COV-2 Infection

PCR (Polymerase Chain Reaction) in Real Time with the PCR Rotor-Gene Q Zybio Kit from China Company was used to identify SARS-COV-2 RNA samples from the nasopharyngeal swap. By assessing the intensity of fluorescent signals during RT-PCR amplification with specific primers and probes against the preserved regions of the N, RdRP, and s genes, this product qualitatively identified SARS-CoV-2 RNA in the samples.

The RNA was isolated using a Kit for extracting viral nucleic acids (Magnetic Bead Method) from the Zybio (China) firm, following the kit's instructions (Magnetic Bead Method) (YXB20180096). Manual, semi-automated, and automatic techniques are provided for producing a sample volume of 200 µL is highly recommended for SARS-CoV-2 RNA extraction and processing. Negative Control for SARSCOV-2 and Positive Control for SARS-CoV-2 both have nucleic acid extraction going on at the same time. Ten microliters of Negative Control for SARSCOV-2 and Positive Control for SARS-CoV-2 nucleic acid samples were added to each PCR reaction tube with filter tips. The number of cycles and temperature of the steps were recorded in a fluorescent PCR apparatus, and the following PCR amplification settings were selected (using the ABI 7500 as an example) (Table 1).

Table 1. The thermal cycling conditions in Real-Time PCR of SARS-COV-2 infection

Steps	Temperature	Time	cycle
UNG reaction	37°C	1 min	1
Reverse transcription	50°C	5 min	1
Initial denaturation	95°C	2 min	1
Denaturation	95°C	5 sec	45
Amplification and fluorescence detection	60°C	30 sec	

Report Fluorescence Setting: FAM, ROX, CY5, Vic, Quenching Fluorescence Setting None, Passive Reference Setting None. Fluorescence Detection: Step 5: Fluorescence Setting: FAM, ROX, CY5, Vic, Quenching Fluorescence Setting None, Passive Reference Setting None. After the reaction, the results were immediately stored, and the target and standard internal curves were examined individually. The baseline's Start, End, and Threshold values were changed based on the picture analysis, and the amplification curve of the negative control was made straight or below the threshold line.

2.3. Statistical Analysis

The Statistical Analysis System - SAS (2012) (12) application was utilized to determine the influence of different components in research parameters. The T-test and the Least Significant Difference – LSD test (Analysis of Variation-ANOVA) were used to make a meaningful comparison between means. This study utilized the Chi-square test for SPSS version 18 (p-value<0.05).

3. Results and Discussion

In this study, COVID-19 infection in 100 Iraqi patients was classified depending on the severity of their infection (mild or moderate, severe and critical) involving males and females between the ages of 25 and 92 years (Table 2), between November 2021 and March 2022. Based on a nasopharyngeal swab, SARS-CoV-2 was identified in all samples using real-time PCR. AFIAS D-Dimer and Ferritin test used a sandwich immunodetection method. Immunometric test using NycoCard CRP sandwich format. There was a statistical difference (high significant difference) (p-value <0.001) between the patient and the control group (Table 3).

Table 2. Patients were divided into three groups based on the severity of the condition

Type of Severity	No	Percentage (%)
Mild or Moderate	34	34.00 %
Sever	33	33.00 %
Critical	33	33.00 %
Total	100	100%
P-value	---	0.997 NS

NS: No-Significant

Table 3. Comparison of Ferritin, D. Dimer, and C-reactive protein between patients and control groups

Group	Mean ± SE		
	Ferritin (ng/ml)	D. Dimer (µg/ml)	C-reactive protein (mg/ l)
Patients	432.13±39.34	3.19 ±0.42	40.37 ±6.44
Control	45.88±2.83	0.234 ±0.012	0.235 ±0.01
T-test	93.718 **	1.009 **	15.339 **
P-value	0.0001	0.0001	0.0001

** (P≤0.01)

Ferritin levels increased significantly with varying degrees of severity, with extremely substantial increases (*p*-value <0.001) between the severity group, mean ± SE (92.45±13.83) in the mild or moderate group, (545.58 ± 57.71) in the severe group while (668.64 ± 71.52) in critical group (Table 4). According to current research, increased ferritin levels might be noticed during the acute phase of a reaction, and when a cytokine storm occurs, it may have a role in inflammation (13). In extreme instances, serum ferritin levels were found to be much higher. A prior investigation found that non-survivors had greater ferritin levels, which matched our findings (14). In addition, ferritin levels were shown to grow in direct proportion to the severity of the illness (15).

Table 4. Effect of type of severity in parameters study of patients

Type of Severity	Mean ± SE		
	Ferritin (ng/ml)	D. Dimer (µg/ml)	C-reactive protein (mg/l)
Mild or Moderate	92.45 ±13.83	0.358 ±0.03	0.450 ±0.07
Sever	545.58±57.71	3.93 ±0.79	25.60 ±4.68
Critical	668.64±71.52	5.36 ±0.79	96.27 ±14.55
LSD value	148.96 **	1.794 **	24.578 **
P-value	0.0001	0.0001	0.0001

* (P≤0.01)

Furthermore, serum ferritin levels greater than 300 ng/mL were associated with an increased mortality risk than ferritin levels less than 300 ng/mL (16). Cao, Wu (17) revealed that ferritin levels cut off 272.5 ng/mL had a sensitivity of 96% and a specificity of 70% (AUC=0.873) in predicting illness severity on admission. As seen in table 5, there was no significant influence of gender on the parameters.

Table 5. Effect of gender in parameters study of patients

Gender	Mean ± SE		
	Ferritin (ng/ml)	D. Dimer (µg/ml)	C-reactive protein (mg/ l)
Male	383.66 ±47.93	3.56 ±0.65	36.81 ±7.24
Female	480.59 62.14	2.84 ±0.54	43.93 ±10.72
T-test	121.61 NS	1.985 NS	20.066 NS
P-value	0.116	0.322	0.651

NS: No-Significant

As shown in table 6, the influence of age on parameters is quite significant. CRP Mean SE age < 40 (8.01± 6.32), age 40-60 (20.93 4.65), age > 60 (67.52 ± 11.90), D-dimer Mean SE age < 40 (1.87 ± 0.96), age 40-60 (2.31±0.66), age > 60 (4.35±0.62), Ferritin Mean SE age < 40 (126.29±40.17), age 40-60 (391.36±60.61), age > 60 (585.25±59.74).

Table 6. Effect of age in parameters study of patients

Age group (year)	Mean ± SE		
	Ferritin (ng/ml)	D. Dimer (µg/ml)	C-reactive protein (mg/ l)
<40	126.29 ±40.17	1.87 ±0.96	8.01 ±6.32
40-60	391.36 ±60.61	2.31 ±0.66	20.93 ±4.65
>60	585.25 ±59.74	4.35 ±0.62	67.52 ±11.90
LSD value	159.58 **	1.693 **	26.331 **
P-value	0.0001	0.0001	0.0001

** (P≤0.01)

As stated by various studies, the SARS-CoV-2 infection has a significant fatality rate, particularly among elderly individuals with comorbid conditions (5, 21). In some cases, the predicted rise in severity with age has been observed. According to estimations, a typical age range is 50 to 60 years (28). Patients above the age of 60, according to Chen, Liu (3), are more likely to have respiratory failure. They revealed that COVID-19 patients in their senior years had a more severe disease than those in their earlier years. The current study also discovered that COVID-19 individuals in the age range (of 50 – 60) years tended to be more severe than younger people, even though numerous laboratory and biochemical characteristics between the (mild-moderate) and (severe – critical) groups were significantly different. CRP, D-dimer, and

ferritin levels were shown to be pathologically increased in older individuals compared to younger patients (23). The current study discovered a substantial rise in Ferritin, D-dimer, and CRP (C-reactive protein) levels in patients with varying degrees of severity (mild or moderate, severe, critical) compared to a control group. The effect of age is highly significant in all parameters, whereas the effect of gender is not significant in any of the patients.

Authors' Contribution

Study concept and design: Z. A. A.
Acquisition of data: L. F. A.
Analysis and interpretation of data: Z. A. A.
Drafting of the manuscript: L. F. A.
Critical revision of the manuscript for important intellectual content: L. F. A.
Statistical analysis: Z. A. A.
Administrative, technical, and material support: Z. A. A.

Ethics

The Ethics Committee, Department of Biology, CSEC/0122/0020, authorized this study on January 20, 2022.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med. 2020;382(10):970-1.
2. Xu C, Luo X, Yu C, Cao S-J. The 2019-nCoV epidemic control strategies and future challenges of building healthy smart cities. SAGE Publications Sage UK: London, England; 2020. p. 639-44.
3. Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. J Med Virol. 2020;92(4):418-23.
4. Hotez PJ, Bottazzi ME, Corry DB. The potential role of Th17 immune responses in coronavirus immunopathology and vaccine-induced immune enhancement. Elsevier; 2020. p. 165-7.
5. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, et al. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses. 2020;12(4):372.
6. De Haan CA, Kuo L, Masters PS, Vennema H, Rottier PJ. Coronavirus particle assembly: primary structure requirements of the membrane protein. J Virol. 1998;72(8):6838-50.
7. Woo PC, Huang Y, Lau SK, Yuen K-Y. Coronavirus genomics and bioinformatics analysis. Viruses. 2010;2(8):1804-20.
8. Al-Suhail RGA, Ali LF. Statistical Analysis of COVID-19 Pandemic Across the Provinces of Iraq. Iraqi J Sci. 2021:811-24.
9. Ghahramani S, Tabrizi R, Lankarani KB, Kashani SMA, Rezaei S, Zeidi N, et al. Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: a systematic review and meta-analysis. Eur J Med Res. 2020;25(1):1-10.
10. Salehi-Abari I, Khazaeli S, Salehi-Abari F, Salehi-Abari A. Practical guideline for screening the patients with SARS-CoV-2 infection and Persian Gulf criteria for diagnosis of COVID-19. Adv Infect Dis. 2020;10(03):67.
11. Yang A-P, Liu J-p, Tao W-q, Li H-m. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol. 2020;84:106504.
12. Cary N. Statistical analysis system, User's guide. Statistical. Version 9. SAS Inst Inc USA. 2012.
13. Kernan KF, Carcillo JA. Hyperferritinemia and inflammation. Int Immunol. 2017;29(9):401-9.
14. Taneri PE, Gómez-Ochoa SA, Llanaj E, Raguindin PF, Rojas LZ, Roa-Díaz ZM, et al. Anemia and iron metabolism in COVID-19: a systematic review and meta-analysis. Eur J Epidemiol. 2020;35(8):763-73.
15. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol. 2020;146(1):110-8.
16. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.

17. Cao P, Wu Y, Wu S, Wu T, Zhang Q, Zhang R, et al. Elevated serum ferritin level effectively discriminates severity illness and predicts prognosis of COVID-19 patients. 2020.
18. Tang N, Li D, Wang X, Sun Z. Des paramètres de coagulation anormaux sont associés à un mauvais pronostic chez les patients atteints de pneumonie à nouveau coronavirus. J Thromb Haemost. 2020;18(4):844-7.
19. Querol-Ribelles JM, Tenias JM, Grau E, Querol-Borras JM, Climent JL, Gomez E, et al. Plasma d-dimer levels correlate with outcomes in patients with community-acquired pneumonia. Chest. 2004;126(4):1087-92.
20. Snijders D, Schoorl M, Schoorl M, Bartels PC, van der Werf TS, Boersma WG. D-dimer levels in assessing severity and clinical outcome in patients with community-acquired pneumonia. A secondary analysis of a randomised clinical trial. Eur J Intern Med. 2012;23(5):436-41.
21. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507-13.
22. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ. 2020;368.
23. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382(18):1708-20.
24. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol. 2020;92(7):791-6.
25. Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, et al. Clinical characteristics of refractory coronavirus disease 2019 in Wuhan, China. Clin Infect Dis. 2021;73(11):e4208-e13.
26. Luo X, Zhou W, Yan X, Guo T, Wang B, Xia H, et al. Prognostic value of C-reactive protein in patients with coronavirus 2019. Clin Infect Dis. 2020;71(16):2174-9.
27. Wang G, Wu C, Zhang Q, Wu F, Yu B, Lv J, et al., editors. C-reactive protein level may predict the risk of COVID-19 aggravation. Open forum infectious diseases; 2020: Oxford University Press US.
28. Verity R, Okell LC, Dorigatti I, Winskill P, Whittaker C, Imai N, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. Lancet Infect Dis. 2020;20(6):669-77.