Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Hematological, immunological, and inflammation markers in patients of COVID-19

Fakhir Najim K. Sabir[1], Edrees Mohammad Ameen[2*]

[1] Department of Biology, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region - F.R. Iraq
[2] Department of Biology, College of Science, Salahaddin University, Erbil, Iraq

ARTICLE INFO

Original paper

Article history:
Received: December 17, 2022
Accepted: February 19, 2023
Published: February 28, 2023

Keywords:

COVID-19; Ferritin; IgG; IgM; CRP; CBC.

ABSTRACT

The present study was done to evaluate hematological, immunological, and inflammation markers in male and female patients infected with COVID-19, ranging from moderate to severe cases in Erbil city of Iraq. The study included 200 samples (60 male, and 60 female patients infected with COVID-19). While 40 healthy males and 40 healthy females were used as a control group. The results found significant differences in total white blood cells (WBC), lymphocytes, immunoglobin G (IgG), immunoglobin M (IgM), C-reactive protein (CRP), Ferritin, and erythrocyte sedimentation rate (ESR) between healthy control and patients infected with COVID-19 in males and females. Patients with COVID -19 showed a significantly $p \leq 0.001$ higher value of total WBC, IgG, IgM, CRP, Ferritin, and ESR when compared with the control group in both males and females. The percentage of lymphocytes in male and female patients is significantly $p \leq 0.001$ lower than that of the healthy control group. No significant differences in red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), and thrombocytes were observed between the control and patient groups in both males and females.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.2.13

Introduction

In December 2019, numerous patients in Wuhan City, Hubei Province, Central China, developed pneumonia of unknown origin. This pneumonia, dubbed coronavirus disease 2019 (COVID-19), is caused by a new CoV, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), formerly known as 2019 novel coronavirus (2019-nCoV) (1-3)

Hematological alterations can and do occur, with the potential to improve the surveillance of infectious processes to signal the severity of their suspicion. Patients initially experience symptoms such as fever, dry cough, exhaustion, and shortness of breath, as well as certain less common symptoms such as diarrhea and vomiting, after being exposed to SARS-CoV-2. Even though most patients with COVID-19 have mild to moderate symptoms, the condition can have serious consequences. Older persons and people with pre-existing chronic medical illnesses like cardiovascular disease, diabetes, and cancer may have a worse clinical phenotype (4, 5). Laboratory abnormalities, particularly hematological alterations, enable monitoring of the status of SARS-CoV-2 infection since the hematopoietic system and hemostasis encounter significant implications throughout the evolution of COVID-19 (6).

Lymphocytopenia, neutrophilia, eosinopenia, moderate thrombocytopenia, and thrombocytosis are the most prevalent hematological abnormalities. The leukocyte count could be normal, low, or high. Neutrophils are the most distinctive WBC type and an essential component of the immune system (7-9).

Antibodies to both IgM and IgG could provide insight into the progression of virus infection (10, 11). IgM is detectable 3–6 days after a SARS infection, and IgG is detectable after 8 days (12). Anti-SARS-CoV-2 serological assays for virus-specific IgM and IgG antibodies have just recently been developed and one COVID-19 patient had similar serological responses (13, 14). The ESR is a key indicator of immunological decline. COVID-19, on the other hand, is a highly infectious disease caused by SARS-CoV-2, and the mechanism of how the virus interacts with the immune system once it infects the human body remains unknown, despite extensive research into the virus's structure and genetic sequence (15)

The present study aimed to evaluate the detection of some hematological (RBC, Hb, WBC, PCV, and thrombocyte), immunological (IgG and IgM) parameters, and inflammation markers (CRP, ferritin, and ESR) in moderate to severe patients infected with COVID-19.

Materials and Methods

Subjects

The present study includes 200 subjects aged between 40-60 years, chosen from Masif clinical lab staff, Floria clinical lab, Santi life hospital, Zheen international hospital, and Shakib clinical lab staff in Erbil city. Four groups were created from the subjects as follows:

Group I (40 healthy males used as a control group).
Group 2 (40 healthy females used as a control group).
Group 3 (60 patient males infected with COVID -19).
Group 4 (60 patient females infected with COVID -19).

The mean ages of the subjects in all groups ranged between 40-60 years with no significant differences between the groups. The mean ages of the male control group were (45.34 ± 10.87); control females (48.46 ±

* Corresponding author. Email: edrees.ameen@su.edu.krd

9.67), males covid-19 (48.54 ± 8.75), and females covid-19 (50.44 ± 7.68). The study has been conducted during the period from March 2021 to September 2022. The patients are infected in moderately to a severe state. The samples were taken during 15-30 days of infection. Smoking, alcoholism, diabetes, lung disease, hypertension, and another disease that interferes with the results are excluded from the study.

Collection of samples

A vein was punctured to take 5 ml of blood from each subject. A 2 ml and a 3 ml portion of the blood sample was divided. To estimate the ESR and conduct a CBC count, the first component was put in a tube containing EDTA. For the second component, the serum was collected by centrifuging it at 3000 rpm for 15 minutes. IgG, IgM, CRP, and ferritin were all tested on the serum after it had been kept at -20 ^{o}C.

Complete blood count

The RBC, Hb, hematocrit (HCT), WBC, lymphocytes, and thrombocytes were measured by using a Coulter Counter instrument (Medonic, USA).

Immunological parameters

IgG and IgM were measured by using the enzyme-linked immunosorbent assay (ELISA).

Inflammation biomarkers

Serum CRP was measured by using Cobas c111, while serum ferritin was estimated by using Cobas e411. ESR was estimated by using of Westergren tube and method.

Statistical analysis

The data was analyzed with the help of SPSS (Version 17). The results are presented as means with standard errors. The analyzed parameters between control and patients of COVID -19 were compared using an independent t-test. A p-value equal to or less than 0.05 was considered to be statistically significant.

Results

Hematological parameters

Comparison of some hematological parameters between patients infected with COVID-19 and healthy control groups are presented in tables 1 and 2. The results found significant differences in total WBC, and lymphocytes between healthy control and patients infected with COVID-19 in males and females. Patients with COVID-19 showed a significantly p≤ 0.001 higher value of total WBC (12.34 ± 0.70 in males and 11.50 ± 0.69 ×10^3/µL in females when compared with the control group in both males (7.68 ± 0.84 ×103/µL) and females (8.68 ± 0.76×103/µL). The percentage of lymphocytes in males 7.32 ± 0.65% and females 14.20 ± 2.24% patients is significantly p≤ 0.001 lower than that of the healthy control groups (32.18 ± 3.12% in males and 31.44 ± 3.65% in females). No significant differences in RBC, Hb, HCT, and thrombocytes were observed between the control and patient groups in both males and females.

Immunological and inflammation markers

Comparison of some immunological and inflammation biomarkers between patients infected with COVID-19 and healthy control groups are presented in tables 3 and 4. The results found significant differences in IgG, IgM, CRP, ferritin, and ESR between healthy control and patients infected with COVID-19 in males and females. About the immunological parameters, patients with covid-19 showed significantly p≤ 0.001 higher value of IgG (28.32 ± 5.10 in males and 36.78 ± 6.32 g/L in females), IgM (11.42 ± 3.69

Table 1. Means ± standard errors of hematological parameters in control and infected males with COVID-19.

Hematological parameters	Control	Patients with covid-19	p-value
RBC (× 10^6/µL)	4.90 ± 0.32	4.78 ± 0.27	0.070
Hb (mg/dL)	15.36 ± 1.45	14.67 ± 1.36	0.065
HCT (%)	44.60 ± 4.76	41.46 ± 3.86	0.068
Total WBC (× 10^3/µL)	7.68 ± 0.84	12.34 ± 0.70	0.001
Lymphocytes (%)	32.18 ± 3.12	7.32 ± 0.65	0.001
Thrombocyte (× 10^3/µL)	248.43 ± 2.12	230.42 ± 28.34	0.058

p-value ≤ 0.05 is considered significant.

Table 2. Means ± standard errors of hematological parameters in control and infected females with COVID-19.

Hematological parameters	Control	Patients with covid-19	p-value
RBC (× 10^6/µL)	4.38 ± 0.36	4.20 ± 0.24	0.060
Hb (mg/dL)	13.40 ± 1.56	12.70 ± 1.46	0.068
HCT (%)	40.28 ± 3.42	38.70 ± 3.42	0.056
Total WBC (× 10^3/µL)	8.68 ± 0.76	11.50 ± 0.69	0.001
Lymphocytes (%)	31.44 ± 3.65	14.30 ± 2.24	0.001
Thrombocyte (× 10^3/µL)	275.46 ± 35.37	268.54 ± 30.26	0.078

p-value ≤ 0.05 is considered significant.

Table 3. Means ± standard errors of some immunological and inflammation biomarkers in control and infected males with COVID-19.

Some immunological and inflammation biomarkers	Control	Patients with covid-19	p-value
IgG (g/L)	7.43 ± 1.25	28.32 ± 5.10	0.001
IgM (g/L)	1.12 ± 0.12	11.42 ± 3.69	0.001
CRP (mg/dL)	0.07 ± 0.01	32.34 ± 4.56	0.001
Ferritin (ng/ml)	130.45 ± 16.17	820.27 ± 90.26	0.001
ESR (mm/hr)	4.34 ± 0.65	65.24 ± 8.89	0.001

p-value ≤ 0.05 is considered significant.

Table 4. Means ± standard errors of some immunological and inflammation biomarkers in control and infected females with COVID-19.

Some immunological and inflammation biomarkers	Control	Patients with covid-19	p-value
IgG (g/L)	8.45 ± 1.67	36.78 ± 6.32	0.001
IgM (g/L)	0.67 ± 0.13	9.27 ± 1.45	0.001
CRP (mg/dL)	0.05 ± 0.01	42.36 ± 6.78	0.001
Ferritin (ng/ml)	94.67 ± 15.36	720.56 ± 75.68	0.001
ESR (mm/hr)	10.34 ± 0.98	72.54 ± 12.68	0.001

p-value ≤ 0.05 is considered significant.

in males and 9.27 ± 1.45 g/L in females) when compared with the control group in males (IgG, 7.43 ± 1.25 and IgM, 1.12 ± 0.12 g/L) and females (IgG, 8.45 ± 1.67 and IgM, 0.67 ± 0.13 g/L). Regarding the inflammation biomarkers, patients with covid-19 showed significantly p≤ 0.001 higher concentrations of CRP (32.34 ± 4.56 in males and 42.36 ± 6.78 mg/dL in females), Ferritin (820.27 ± 90.26 in males and 720.56 ± 75.68 ng/ml in females) and ESR (65.24 ± 8.89 in males and 72.54 ± 12.26 mm/hr in females) when compared with the control group in males (CRP, 0.07 ± 0.01 mg/dL, Ferritin, 130.45 ± 16.17 ng/ml and ESR 4.34 ± 0.65 mm/hr) and females (CRP, 0.05 ± 0.01 mg/dL, Ferritin, 94.76 ± 15.36 ng/ml and ESR 10.34 ± 0.98mm/hr).

Discussion

The observed results in the present study are in agreement with the findings of (16) who record lymphopenia in severe COVID-19 disease. Also (17) found that patients in the severe group had considerably ($p<0.05$) fewer lymphocytes than those in the non-severe infected with the COVID-19 group in Chongqing, China. According to recent research, lymphocyte counts in COVID-19 patients with mild illness are normal. On the other hand, lymphopenia is found in 20–96% of severe illnesses (18, 19). Lymphocytes play an important role in both inflammation and infection. While these parameters may be used as inflammatory markers by themselves, their ratios to one another may also be indicators of early inflammation (7-9). The ratio of these two features was used as an inflammatory measure because stress induces circulating leukocytes to increase neutrophils and reduce lymphocytes (20). The results of the present study are in contrast with the finding of (21) who found a drop in hemoglobin and thrombocytes in COVID-19 patients' injured lungs as a result of platelet consumption and/or production reduction.

The recorded results regarding immunological and inflammation markers are in agreement with the findings of (22) who concluded that IgM levels rose during the first week after SARS-CoV-2 infection, peaked after two weeks, and subsequently fell to near-background levels in the majority of patients. After one week, IgG was detected and remained at a high level for a long time. The rates of IgM and/or IgG antibody detections in the mild, severe, and critical illness categories were not substantially different. IgM levels were greater in severe and critical cases than in mild cases, but IgG levels were lower in critical cases than in both mild and severe cases. IgM is detectable 3–6 days after a SARS infection, and IgG is detectable after 8 days (23). Anti-SARS-CoV-2 serological assays for virus-specific IgM and IgG antibodies have just recently been developed and one COVID-19 patient had similar serological responses (13, 14). Quick and specific antibody detection could provide information for confirmation or exclusion of SARS-CoV-2 infection in suspected patients, according to the Chinese National Health Commission's current 'Guideline of diagnosis and treatment for COVID-19' (24).

The mentioned results are in agreement with the observation of (25), who found a significant elevation of inflammation markers such as CRP, serum Ferritin, and ESR in patients of COVID-19. Age, gender, and physical condition have little impact on CRP concentration, which is related to the intensity of inflammation (26). The complement system and phagocytosis are activated by CRP levels, which help the body get rid of dangerous microbes Patients with severe pneumonia had higher CRP levels, and CRP levels can be used to detect pneumonia early (27). It's a critical factor for diagnosing and assessing severe lung infectious illnesses (28). Matsumoto's research also demonstrated the importance of CRP levels in cases of severe pneumonia (29). This study found that as the disease progressed, CRP levels and the width of the largest lung lesion increased. The severity of the disease and lung lesions were found to be correlated with CRP levels. This demonstrates that in the early stages of COVID-19, CRP levels may be a good indicator of lung lesions and disease severity. Ferritin, through direct immuno-suppressive and pro-inflammatory actions, contributes to the cytokine

storm and is a significant modulator of immune dysregulation, especially in extreme hyperferritinemia (30). The observation that deadly COVID-19 results are accompanied by cytokine storm syndrome suggests that cytokine storm syndrome affects the severity of the illness (31). Many diabetics have high ferritin levels in their blood, and it's well-recognized that they're more likely to develop major COVID-19 issues (32, 33).

In conclusion, the current study concluded that moderate to severe COVID-19 patients have a significant elevation of lymphocytes and immunological factors such as IgG and IgM. Moreover, the inflammation markers such as serum CRP, ferritin, and ESR also increased.

Acknowledgments
The authors would like to thank all of the participants in the study and all staff in the Santi life hospital, Zheen international hospital, Shakib clinical, Masif clinical lab, and Floria clinical lab in Erbil city.

Conflict interest
The authors declare no conflict of interest.

Authors' Contribution
The design of the study and the statistical analysis was heavily influenced by Edrees Mohammad Ameen. The study and production of the manuscript were equally shared by all authors.

Ethics approval and consent to participate
The samples of the study were taken following the Helsinki Declaration of 1975, as revised in 2000 and approved by the Human Ethical Committee of Koya University, Faculty of Science and Health, Biology department, and numbered sheet 5 issued on 4/7/2021.

References

1. Bai Y, Yao L, Wei T, Tian F, Jin D-Y, Chen L, et al. Presumed asymptomatic carrier transmission of COVID-19. JAMA. 2020;323(14):1406-7.
2. Wong JE, Leo YS, Tan CC. COVID-19 in Singapore—current experience: critical global issues that require attention and action. JAMA. 2020;323(13):1243-4.
3. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci. China Life Sci. 2020;63(3):457-60.
4. Liu W, Zhang Q, Chen J, Xiang R, Song H, Shu S, et al. Detection of Covid-19 in children in early January 2020 in Wuhan, China. N Engl J Med.2020;382(14):1370-1.
5. Shekerdemian LS, Mahmood NR, Wolfe KK, Riggs BJ, Ross CE, McKiernan CA, et al. Characteristics and outcomes of children with coronavirus disease 2019 (COVID-19) infection admitted to US and Canadian pediatric intensive care units. JAMA Pediatrics. 2020;174(9):868-73.
6. Debuc B, Smadja DMJScr, reports. Is COVID-19 a new hematologic disease? Stem Cell Rev Rep. 2021;17(1):4-8.
7. İlhan M, İlhan G, Gök AF, Bademler S, Verit Atmaca F, Ertekin C. Evaluation of neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and red blood cell distribution width-platelet ratio as early predictor of acute pancreatitis in pregnancy. J Matern Fetal Neonatal Med. 2016;29(9):1476-80.
8. Yazar FM, Bakacak M, Emre A, Urfalıoglu A, Serin S, Cengiz E, et al. Predictive role of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios for diagnosis of acute appendicitis during pregnancy. Kaohsiung J Med Sci. 2015;31(11):591-6.
9. Liu J, Li S, Zhang S, Liu Y, Ma L, Zhu J, et al. Systemic immune-inflammation index, neutrophil-to-lymphocyte ratio, the platelet-to-lymphocyte ratio can predict clinical outcomes in patients with metastatic non-small-cell lung cancer treated with nivolumab. J Clin Lab Anal. 2019;33(8):e22964.
10. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3.
11. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol. 2020;92(9):1518-24.
12. Lee HK, Lee BH, Seok SH, Baek MW, Lee HY, Kim DJ, et al. Production of specific antibodies against SARS-coronavirus nucleocapsid protein without cross-reactivity with human coronaviruses 229E and OC43. J Vet Sci. 2010;11(2):165-7.
13. Woo PC, Lau SK, Wong BH, Tsoi H-w, Fung AM, Chan K-h, et al. Detection of specific antibodies to severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein for serodiagnosis of SARS coronavirus pneumonia. Clin Microbiol Newsl.2004;42(5):2306-9.
14. Xiao SY, Wu Y, Liu H. Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol. 2020;92(5):464-7.
15. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33.
16. Pozdnyakova O, Connell NT, Battinelli EM, Connors JM, Fell G, Kim AS. Clinical significance of CBC and WBC morphology in the diagnosis and clinical course of COVID-19 infection. Am J Clin Pathol. 2021;155(3):364-75.
17. Yang H, Xu Y, Li Z, Yan L, Wang J, Liao P. The Clinical Implication of Dynamic Hematological Parameters in COVID-19: A Retrospective Study in Chongqing, China. Int J Gen Med. 2021;14:4073.
18. Alhazzani W, Møller M, Arabi Y, Loeb M, Gong M, Fan E. & Du, B.(2020). Surviving Sepsis Campaign: guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). Intensive Care Med.1-34.
19. Ding X, Yu Y, Lu B, Huo J, Chen M, Kang Y, et al. Dynamic profile and clinical implications of hematological parameters in hospitalized patients with coronavirus disease 2019. Clin Chem Lab Med. 2020;58(8):1365-71.
20. Xiang N, Havers F, Chen T, Song Y, Tu W, Li L, et al. Use of national pneumonia surveillance to describe influenza A(H7N9) virus epidemiology, China, 2004-2013. Emerg Infect Dis.2013;19(11):1784-90.
21. Liu X, Zhang R, He G. Hematological findings in coronavirus disease 2019: indications of progression of disease. Ann Hematol. 2020;99:1421-8.
22. Hou H, Wang T, Zhang B, Luo Y, Mao L, Wang F, et al. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. Clin Transl Immunol. 2020;9(5):e1136.
23. Lee H-K, Lee B-H, Seok S-H, Baek M-W, Lee H-Y, Kim D-J, et al. Production of specific antibodies against SARS-coronavirus nucleocapsid protein without cross-reactivity with human coronaviruses 229E and OC43. J Vet Sci. 2010;11(2):165.
24. Committee GOoNH. Office of state administration of traditional Chinese medicine. Notice on the issuance of a program for the diagnosis and treatment of novel coronavirus (2019-nCoV) infec-

ted pneumonia (trial version 6).Text in Chinese. 2020.
25. Fei F, Smith JA, Cao L. Clinical laboratory characteristics in patients with suspected COVID-19: One single-institution experience. J Med Virol. 2020.
26. Pan F, Yang L, Li Y, Liang B, Li L, Ye T, et al. Factors associated with death outcome in patients with severe coronavirus disease-19 (COVID-19): a case-control study. Int J Med Sci 2020;17(9):1281.
27. Grasselli G, Pesenti A, Cecconi M. Critical care utilization for the COVID-19 outbreak in Lombardy, Italy: early experience and forecast during an emergency response. JAMA. 2020;323(16):1545-6.
28. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med. 2020;46(6):1089-98.
29. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4.
30. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci. 2014;19(2):164-74.
31. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
32. Momeni A, Behradmanesh MS, Kheiri S, Abasi F. Serum ferritin has a correlation with HbA1c in type 2 diabetic patients. Adv Biomed Res. 2015;4:74.
33. Son NE. Influence of ferritin levels and inflammatory markers on HbA1c in the Type 2 Diabetes mellitus patients. Pak J Med Sci Q. 2019;35(4):1030-5.