ORIGINAL ARTICLE

Hyperferritinemia as a factor associated with poor prognosis in COVID-19 patients

Hyperferitinemie jako faktor spojený se špatnou prognózou u pacientů s COVID-19

Nuhad Mohammed Al-dulaimi • Mahmood Jassim Mohammed • Saad T. Mutlk • Khalid F. Al-rawi • Hameed Hussein Ali • Bilal J. M. Aldahham • Faisal Al-ani • Osamah Al-ani • Yaqout A. Hamed • Aus T. Ali

Received June 12, 2023 / Accepted August 15, 2023

Summary

Worldwide, hundreds of millions of people have been infected with COVID-19 since December 2019; however, about 20% or less developed severe symptoms. The main aim of the current study was to assess the relationship between the severity of Covid-19 and different clinical and laboratory parameters. A total number of 466 Arabs have willingly joined this prospective cohort. Out of the total number, 297 subjects (63.7%) had negative COVID-19 tests, and thus, they were recruited as controls, while 169 subjects (36.3%) who tested positive for COVID-19 were enrolled as cases. Out of the total number of COVID-19 patients, 127 (75.15%) presented with mild symptoms, and 42 (24.85%) had severe symptoms. The age range for the participants was 20 to 82 years. Compared with controls, the severity of the disease was associated with significantly high ferritin levels ($P < 0.001$). The severity of the disease was also associated with a significant increase in C-reactive protein ($P < 0.001$), D-dimer ($P < 0.001$), white blood cell count (WBC) ($P < 0.01$), IgM ($P < 0.001$), and Granulocytes ($P < 0.01$). In addition, severe COVID-19 symptoms in the current study were associated with a significant decrease in lymphocytes ($P < 0.01$). There was a four-fold increase in serum ferritin levels in COVID-19 patients presented with severe symptoms upon admission. The former was associated with significantly high levels of CRP and D-dimer. Thus, hyperferritinemia, together with high CRP and D-dimer concentrations, may serve as reliable predictors for disease severity and poor prognosis in Arabs with COVID-19.

Key words: COVID-19 • ferritin • mild symptoms • severe symptoms • poor prognosis

Nuhad Mohammed Al-dulaimi
General Directorate of Education in Anbar, Ministry of Education, Al-anbar Province, Iraq

Mahmood Jassim Mohammed
Anbar Health Department, Ministry of Health, Al-anbar Province, Iraq

Saad T. Mutlk
Department of Biology, College of Science, University of Anbar, Iraq

Khalid F Al-rawi • Hameed Hussein Ali
Department of Chemistry, College of Science, University of Anbar, Al-anbar Province, Iraq

Bilal J. M. Aldahham
Department of Applied Chemistry, College of Applied Sciences, University of Anbar, Al-anbar Province, Iraq

Faisal Al-ani • Osamah Al-ani (✉)
Faculty of Medicine, Odessa National Medical University
Odessa 65000, Ukraine
e-mail: oalani@europe.com

Yaqout A. Hamed
College of Pharmacy, University of Baghdad

Aus T. Ali
Faculty of Medicine, Odessa National Medical University, Odessa, Ukraine
Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Souhrn

Od prosince 2019 se onemocněním COVID-19 nakazily stovky milionů lidí na celém světě, avšak závažné příznaky se objevily u 20 % nebo méně. Hlavním cílem této studie bylo posoudit vztah mezi závažností onemocnění COVID-19 a různými klinickými a laboratorními parametry. Do tohoto prospektivního souboru se ochotně zapojilo celkem 466 Arabů. Z celkového počtu mělo 297 osob (63,7 %) negativní test na COVID-19, a byly tedy zařazeny jako

kontroly, zatímco 169 osob (36,3 %), u nichž byl test na COVID-19 pozitivní, bylo zařazeno jako případy. Z celkového počtu pacientů s COVID-19 mělo 127 pacientů (75,15 %) mírné příznaky a 42 pacientů (24,85 %) mělo závažné příznaky. Věkové rozmezí účastníků bylo 20 až 82 let. V porovnání s kontrolní skupinou byla závažnost onemocnění spojena s významně vyššími hladinami feritinu ($P < 0,001$). Závažnost onemocnění byla rovněž spojena s významným zvýšením C-reaktivního proteinu ($P < 0,001$), D-dimerů ($P < 0,001$), počtu bílých krvinek (WBC) ($P < 0,01$), IgM ($P < 0,001$) a granulocytů ($P < 0,01$). Kromě toho byly závažné příznaky COVID-19 v této studii spojeny s významným poklesem lymfocytů ($P < 0,01$). U pacientů s COVID-19, kteří se při přijetí prezentovali závažnými příznaky, došlo ke čtyřnásobnému zvýšení hladiny feritinu v séru. Ten byl spojen s významně vyššími hladinami CRP a D-dimerů. Hyperferitinémie spolu s vysokými koncentracemi CRP a D-dimerů tak mohou sloužit jako spolehlivé prediktory závažnosti onemocnění a špatné prognózy u Arabů s COVID-19.

Klíčová slova: COVID-19 • feritin • mírné příznaky • závažné příznaky • špatná prognóza

Introduction

The sudden outbreak of the Coronavirus in 2019 (COVID-19) has caused an unimaginable shockwave across the world. It has spread rapidly from China to every corner of the globe, resulting in a disastrous increase in the number of infections and deaths[1]. Complications associated with severe symptoms involve severe respiratory syndrome, assisted mechanical ventilation, and increasing death incidence rate[1, 2]. Clinical features of patients with severe COVID-19 symptoms include a significant decrease in lymphocytes[3–5] which is usually associated with a significant increase of C-reactive protein, D-dimer, IgM[6], white blood cell count (WBC), granulocytes[7, 8], and ferritin levels[1, 2]. Nevertheless, the prognostic role of these biomarkers in COVID-19 patients is ambiguous. Studies regarding the importance of D-dimer as a potential biomarker in patients with COVID-19 are conflicting as some reported significant correlation with poor outcomes[1, 2, 9], while others showed such a relationship is limited only to venous thromboembolism events[10]. Although, these results have limited D-dimer advantages as reliable biomarker in different COVID-19 scenarios, however it continued to be used to screen for patients with venous thromboembolism and identifies those at risk[10]. Ferritin was another biomarker that has been targeted during COVID-19 in order to evaluate the fluctuating levels of this biomarker during different disease stages and severity[1, 2].

Clinically, high levels of ferritin or hyperferritinemia has been used as biomarker of uncontrolled inflammation[11]; thereby serum ferritin level was suggested as biomarker to examine the effectiveness of treatment and to monitor patients' response[11–14]. In addition, high ferritin level is considered as an immune-suppressant and hyperferritinemia plays a major role in the immune system's dysregulation, directly as an immune suppressor and indirectly as a vital pro-inflammatory contributor towards cytokine storm[15–17]. In laboratory medicine, ferritin is considered to be one of the most requested tests to evaluate body iron status and the most frequently requested test in laboratory medicine in primary care and referral settings[12, 18]. Ferritin levels are not always seen within the normal range, but low ferritin level is a reliable marker for iron deficiency[13, 18] and mild elevation is commonly reported in clinical practice[13, 14]. It is estimated that around 10% of individuals with hyperferritinemia are due to iron overload, while the rest is due to specific conditions mostly related to inflammation[13]. Previous[1, 2] and most recent studies[15] have observed high ferritin levels in COVID-19 patients presented with severe symptoms. The search for molecular markers for COVID-19 comes in great demand to decrease disease side effects and increase survival rate. It would be essential to elucidate the causes of severe COVID-19 and to identify reliable markers associated with disease severity. Therefore, the current study was designed to evaluate biochemistry alterations in COVID-19-infected people and to examine the possible correlation between clinical features and candidate biochemistry biomarkers.

Materials and methods

In the current study, we have performed a hospital perspective cohort study including 466 participants (169 COVID-19 cases and 297 controls) who willingly joined the study when they were admitted to the Ramadi General Hospital, the province of Al-Anbar, Iraq, between April and December 2020.

Ethical approval

The study protocol was approved ethically by the Scientific Research Ethics Committee at the University of Anbar (No: 123SC-2020), and every participant signed a written consent form after listening to a brief presentation about the project. The study was performed in conformity with the World Medical Association Declaration of Helsinki.

Definition of cases and controls

In the COVID-19 group, only those confirmed by fast test and real-time polymerase chain reaction or quantitative polymerase chain reaction (PCR) as positive cases were enrolled in the current study as cases. Furthermore, all patients were examined using lung ultrasound and chest CT scan. Controls were approached the hospital complaining of symptoms similar to that of COVID-19; however, their tests were negative, as confirmed through clinical examination

by specialists and PCR testing. COVID-19 patients (n = 169) were then divided into two groups based on the disease severity as follows: the first group included 127 COVID-19 patients with mild symptoms, while the critically ill or patients with severe symptoms group included only 42 COVID-19 subjects.

Collecting of the cases

All cases of the current study were collected from Ramadi General Hospital, Ramadi City, Iraq. The severity of the COVID-19 patients was classified into mild and severe based on symptoms upon admission and the need for assisted ventilation, hospitalization, and intubation. While both groups shared symptoms like fever, cough, tiredness, loss of smell and/or taste, headache, bone aches, runny nose, loss of appetite, and some cases reported diarrhea and red, irritated eyes or achy eyes. Besides these symptoms, severe cases were suffering from shortness of breath, chest pain, loss of speech, loss of mobility, and difficulty breathing. All suspected and mild cases were sent home after giving their details, including a full description of their conditions and full contact details to get feedback within 24 hours to confirm their health status after getting the PCR results. These individuals were asked to isolate themselves for the next 24 hours until getting feedback regarding the test's result. All mild cases were given remedies as prescribed in COVID-19 treatments, protocol with complete instruction to manage their symptoms at home. Severe cases were admitted to the hospital instantly, and cases with difficulty breathing were supplied with oxygen. Survived cases stayed from 6 hours to 7 days in the hospital before they were discharged. Some very severe cases were requested to stay home, while clinicians made the urgent visit with an unbalance and brought these patients to hospitals when needed.

Collecting the controls

All controls in this study approached the same hospital where cases were recruited as they were suspected to have COVID-19 and had symptoms suspected to be COVID-19. However, their test results were negative. These results were confirmed after a quick check-up by clinicians at the hospital before going through a PCR test to confirm that they were COVID-19 negative. Following PCR, all controls had to wait 24 hours after performing before officially being enrolled as controls.

Inclusion criteria

Participant living in the province of Al-Anbar, mostly from Ramdi city and outskirt, who had a positive PCR result confirming the infection with COVID-19, were Arab and 20 years or older at the time of the commencement of the study was included as cases in the currents study. Those who had negative PCR results have been included as controls. The age range for cases and controls was 20 to 82 years.

Exclusion criteria

We excluded all individuals with chronic obstructive pulmonary disease and those with a history of liver damage such as cirrhosis, liver failure, hepatitis B virus (HBV), and hepatitis C virus (HCV). Because the study was designed to target Arabs and in order to avoid any interruption with genetic predisposition, we have excluded all other ethnic groups. Finally, individuals above or below the selected age range of the current study were also excluded.

Samples collection and related measurements

Samples from COVID-19 patients were collected at a separate section out of the hospital. Patients then were directed to the next door room which was prepared for blood withdrawal with the help of another nurse. White blood count (WBC), serum values of C-reactive protein (CRP), COVID-IgM, lymphocytes, D-dimer, Gran and ferritin were quantified in all patients and controls enrolled in the study. Serum concentrations of CRP was evaluated using a specific automated protein analyzer (PA120) provided by (Shenzhen Genius Electronics Co., Ltd. China 2019). Measuring IgM was performed using Vidas technique (ELISA) which is designed to measure the titer of IgM. Other biochemistry biomarkers included: complete blood count (CBC**),** measured using the Medonic technique; CRP measured using Biolis (Biochemistry); ferritin and D-dimer measured using the Vidas technique.

Statistical analysis

Collected data were analyzed using analysis of variance (ANOVA) to assess any differences between cases (COVID-19 groups) and controls. Data are expressed as mean ± standard deviation (SD) or *n* (%). As illustrated in Figures 1, 2, 3, 4, 5, and 6, a comparison between COVID-19 patients and their controls has been performed after dividing the COVID-19 group into two sub-groups (Mild and Severe) based on the severity

Table 1. COVID-19 cases of the current study after been divided by age ranges and disease severity

Age range	No. of cases	Mild symptoms	Severe symptoms
20–39	78	69	9
40–59	55	44	11
≥ 60	36	14	22
Total	169	127	42

of the symptoms at time upon admission. Thus, we have divided the cases into two groups: patients who developed mild symptoms (75.15%) and were sent back home and patients with severe symptoms (24. 75%) who had been hospitalized and their health status over the period of hospitalization either improved or worsened. To examine the effect of age progression on disease severity, we have divided COVID-19 cases into three age groups (age ranges; 20 to 39, 40–59, and ≥ 60 years old) as described in Table 1. For continuous variables, differences between two groups or more were assessed by *t*-test or Mann–Whitney test and

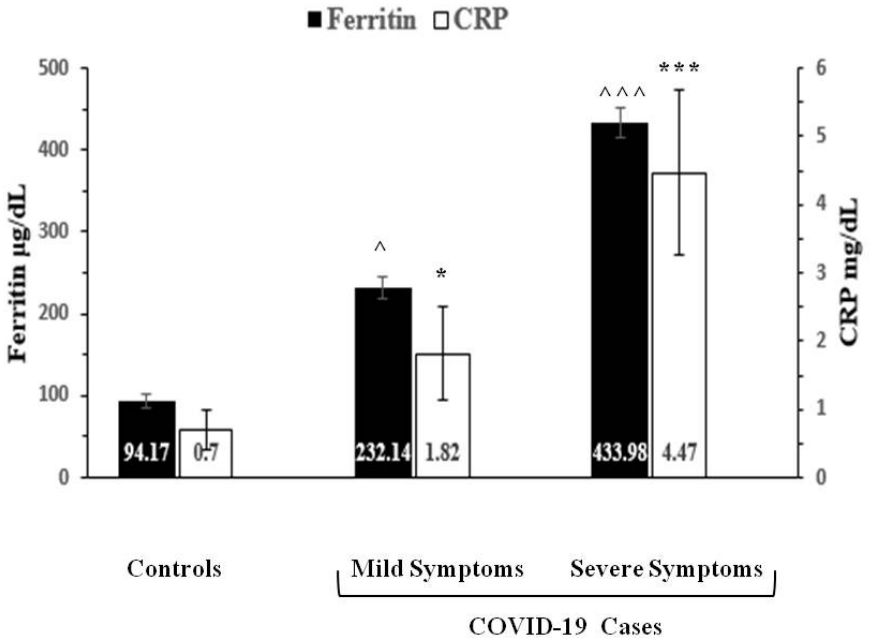

Fig. 1. The relationship between ferritin level and CRP concentrations in patients with COVID-19 and their controls. Compared with controls, a significant elevation in serum ferritin level (P < 0.001) in critically ill patients with COVID-19 was associated with a significant increase in CRP concentrations (P < 0.01).
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$, $^{\wedge}P < 0.05$

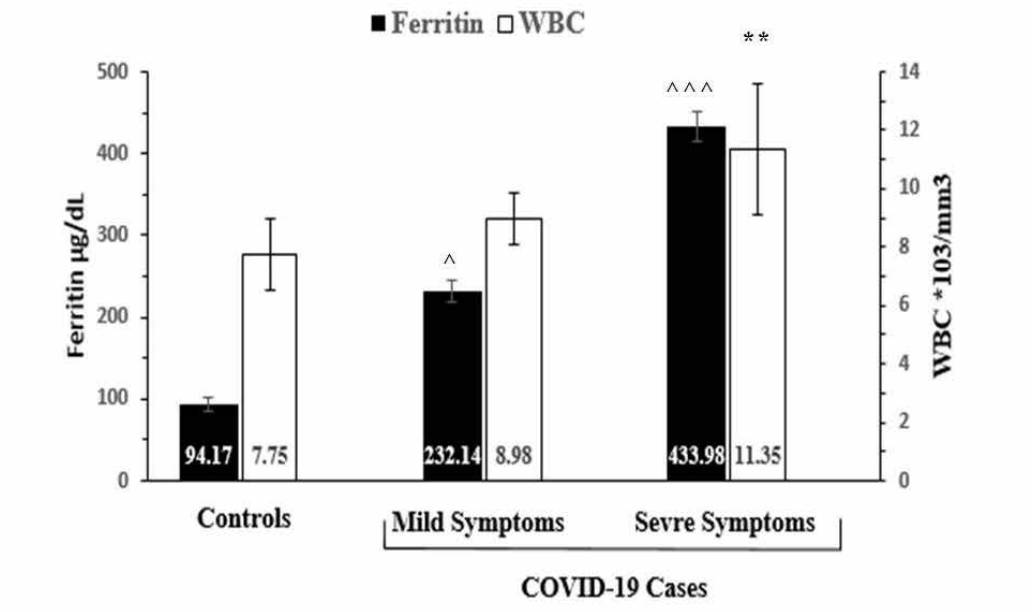

Fig. 2. The relationship between ferritin level and white blood cell count (WBC) in patients with COVID-19 and their controls. Compared with controls, a significant elevation of serum ferritin level (P < 0.001) in critically ill patients with COVID-19 was associated with a significant increase in WBC (P < 0.01).
$^{**}P < 0.01$, $^{\wedge}P < 0.05$, $^{\wedge\wedge\wedge}P < 0.001$

ANOVA or Kruskal–Wallis test with the correspondent post-tests, according to the variable distribution. To compare differences between proportions of categorical variables, we used a two-sided chi-square test. The lowest level of statistical significance was set as long as the *P* value was less than 0.05.

Results

Regrouping COVID-19 patients according to their ages showed a critical increase in the number of severe cases with age (Table 1). Thus, the increasing age of the patients is associated with a sharp increase in the

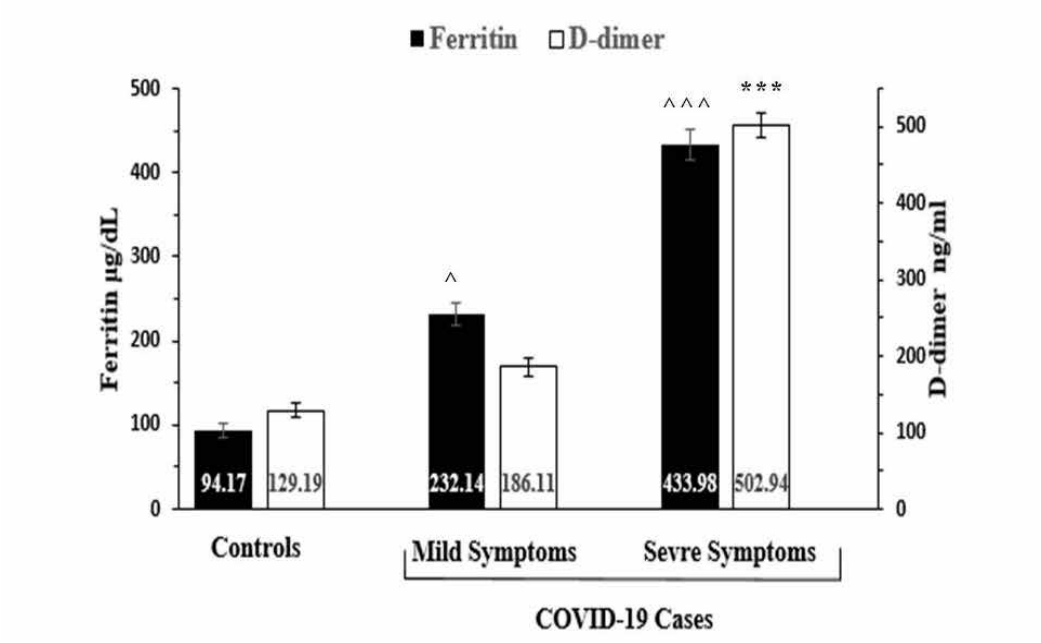

Fig. 3. The relationship between serum ferritin levels and D-dimer concentrations in patients with COVID-19 and their controls. Compared with controls, a significant elevation in serum ferritin level (P < 0.001) in patients with severe COVID-19 was associated with a significant increase in D-dimer concentrations (P < 0.001).
**P < 0.05, **P < 0.01, ^P < 0.05, ^^^P < 0.001*

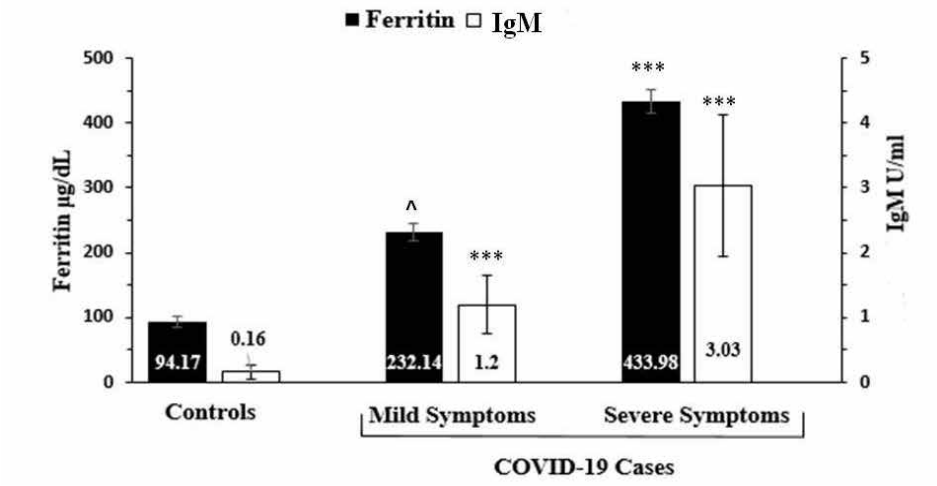

Fig. 4. The relationship between ferritin Level and IgM concentrations in patients with COVID-19 and their controls. Compared with controls, a significant elevation of serum ferritin level (P < 0.001) in critically patients with COVID-19 was associated with a significant increase in IgM concentrations (P < 0.001).
***P < 0.01, ***P < 0.01, ^P < 0.05, ^^^P < 0.001*

probabilities to have poorer outcomes. Compared with controls, the current study has shown a more than four-fold increase in serum ferritin levels in patients with severe COVID-19 ($P < 0.001$). Significant differences were also observed between COVID-19 patients presented with mild symptoms compared with control ($P < 0.01$). The significant increase in serum ferritin level in patients with severe COVID-19 was correlated with a significant increase in CRP level ($P < 0.001$), as shown in Figure 1. Expectedly, there was a significant increase ($P < 0.01$) in WBC in patients with severe COVID-19 compared with the control group, and this was in parallel with

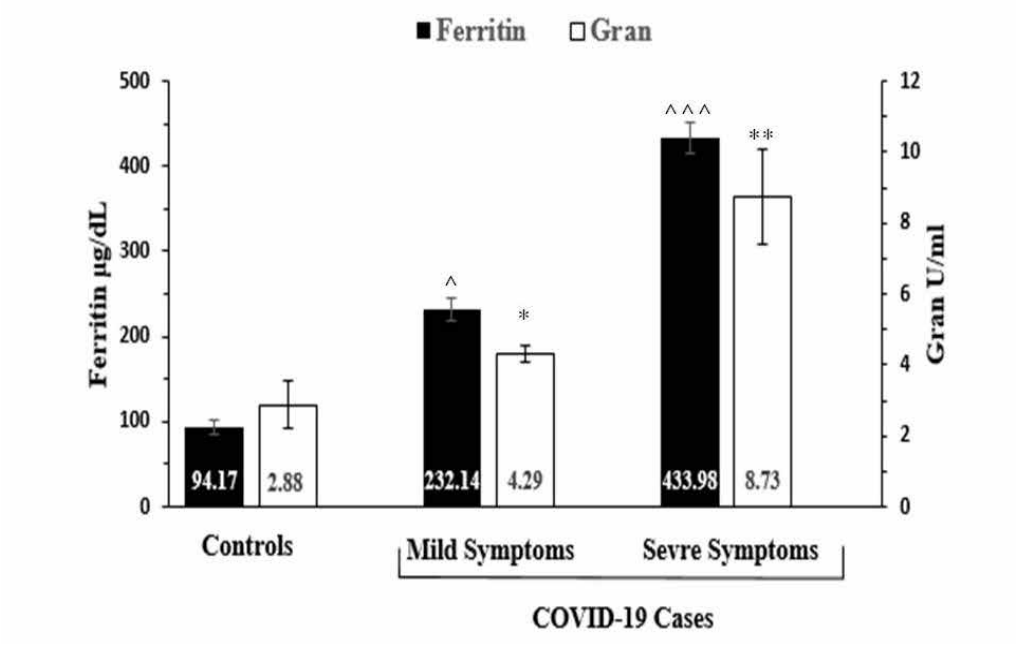

Fig. 5. The relationship between ferritin level and gran concentrations in patients with COVID-19 and their controls. Compared with controls, a significant elevation of serum ferritin level ($P < 0.001$) in critically ill patients with COVID-19 was associated with a significant increase in granulocytes count ($P < 0.01$).
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{\wedge}P < 0.05$, $^{\wedge\wedge\wedge}P < 0.001$

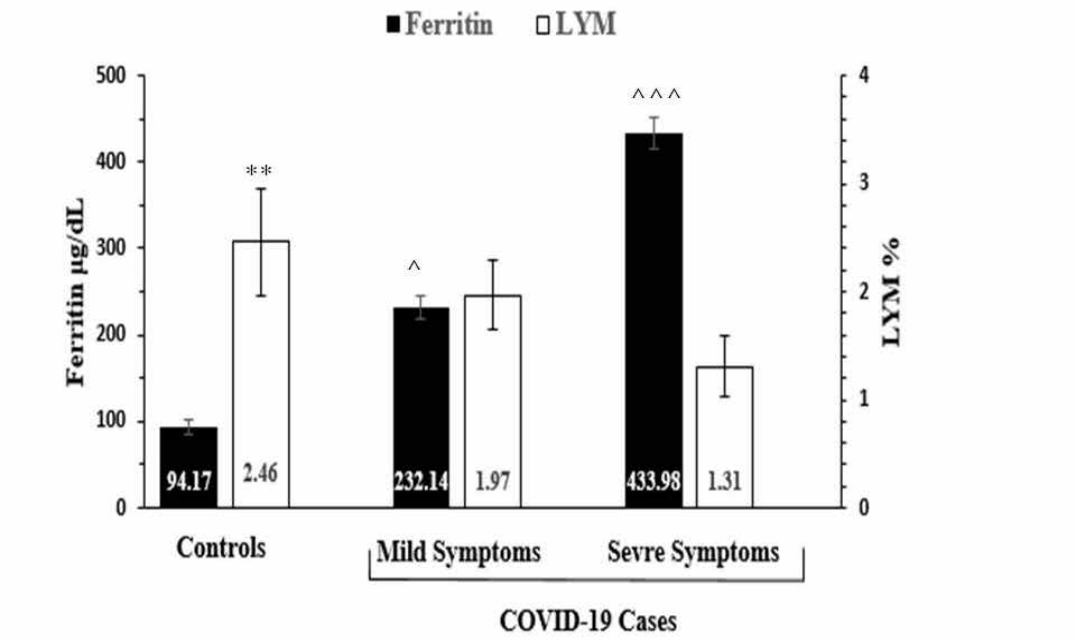

Fig. 6. The relationship between ferritin level and lymphocytes in patients with COVID-19 and their controls. Compared with controls, a significant elevation in serum ferritin level ($P < 0.001$) in patients with COVID-19 was associated with a significant reduction in lymphocytes ($P < 0.01$).
$^{**}P < 0.01$, $^{\wedge}P < 0.05$, $^{\wedge\wedge\wedge}P < 0.001$

the significant increase in serum ferritin level (Fig. 2). Although WBC has increased in patients presented with mild symptoms, this increase was not significant and was within the normal range (Fig. 2). Compared with their counterparts in the control group, as shown in Figure 3, severe COVID-19 patients presented with high serum ferritin levels have witnessed an increase in D-dimer. However, that increase in D-dimer was highly significant in patients who presented with severe symptoms ($P < 0.001$). In accordance with all infectious diseases, there was a significant increase in IgM in patients with severe COVID-19 compared with controls (Fig. 4). These differences were observed in both groups, mild ($P < 0.01$) and severe symptoms group ($P < 0.001$). A significant increase in granulocytes' concentrations was observed in patients presented with severe COVID-19 symptoms compared with controls ($P < 0.01$) as shown in Figure 5. Compared with controls, the significant increase in granulocytes in COVID patients presented with mild symptoms was also detected at lower level of significance ($P < 0.05$).

The significant increase of serum ferritin level in COVID-19 patients presented with severe symptoms was associated with a significant decrease in lymphocytes in comparison with the control group ($P < 0.01$). Although, patients with mild symptoms have witnessed a decrease in lymphocytes compared with their counterpart controls, however these differences were not significant (Fig. 6).

Discussion

The current study has shown that ferritin level is significantly higher in COVID-19 patients than in controls. In addition, when taking into consideration subdividing COVID-19 patients according to the severity of the disease, these differences become even higher when comparing ferritin levels in patients with severe COVID-19 and controls ($P < 0.001$). Hyperferritinemia is frequently observed in COVID patients with severe symptoms, and significant differences was detected between these patients and COVID patients presented with mild symptoms ($P < 0.01$). These results suggest crucial roles of ferritin in the pathogenesis of COVID-19. In addition, increasing the incidence of hyperferritinemia among patients with severe symptoms may indicate a direct relationship between ferritin and disease severity. Nevertheless, when dividing COVID-19 cases by age range (Table 1), we have observed that more than three quarters of the severe cases were 40 years and older, while more than half of the severe cases (52%) were 60 years and older. Furthermore, the number of severe cases in older age groups was more than double compared with similar cases in younger age groups, despite the fact that older age groups have less than half of the total number of the cases that the younger ages had. The significant increase in COVID-19 severity with age progression is in line with previously published data[19–21]. Older age is associated with immune dysregulation due to physiological changes that affect the immune system[19, 21, 22]. A dysregulated immune status occurs when the immune system cannot control or restrain an immune response[21, 22]. This causes a greater susceptibility to infectious disease[19–22]. Compared with younger individuals, previous studies have shown that older COVID-19 patients are in urgent need for hospitalization, admission to the intensive care unit (ICU) and/or mechanical ventilation[19–21]. This group also had the highest death rate due to COVID-19 compared with younger age groups[19–21, 23]. Such deterioration in the immune system contributes crucially not only to autoimmune diseases but also to cardiovascular, metabolic and neurodegenerative diseases of aging[19–23]. The human immune system undergoes crucial aging-related changes and loses the ability to protect against infectious and non-infectious diseases including cardiometabolic diseases and different types of cancer. Consequently, older age has been considered as a risk factor for many diseases including cardio-metabolic diseases[24, 25] and different types of cancers[26–29]. While the COVID-19 pandemic was an indirect risk factor for weight gain and obesity in children[30] and adults[31] due to physical inactivity, sedentary lifestyle, and poor eating patterns, on the other hand, obesity and other cardiometabolic diseases are both associated with increased susceptibility to COVID-19 and worst outcomes[32]. This could be due to the proinflammatory state of cardiometabolic disease and obesity[32].

To our knowledge, this is the first study examining ferritin levels in Arabs with COVID-19 and the relationship between older age and disease severity. In agreement with these findings, previous and most recent studies have shown that COVID-19 is associated with significantly high serum ferritin level upon hospital admission[1, 2]. Furthermore, among those who presented with severe symptoms, serum ferritin levels continue elevating throughout their hospital stay and only exceeded the upper level of detection after 16 days of hospitalization[2].

In another word, ferritin levels continue escalating following the detection of COVID-19 and the highest level of ferritin was observed in those with longer periods in hospital. Most importantly, the highest level of ferritin was associated even with further deterioration in health status of patients diagnosed with severe COVID-19 and was closely related to high death rates[2]. Thus, increases in serum ferritin upon admission of patients with COVID-19 is an excellent predictor of poor prognosis. Unfortunately, in the current study, we did not perform any follow-up to evaluate escalating levels of ferritin as we have relied on one point time test which was upon patients' admission to the hospital. Increasing ferritin levels in patients with COVID-19 is of a high importance and may have a great prognostic value since it is associated with poor prognosis. The higher ferritin level in COVID-19

patients was associated with significant increase in CRP ($P < 0.001$) and D-dimer ($P < 0.001$). Among patients with severe symptoms, there was more than six fold increase in CRP levels compared with controls. Similarly, increased ferritin level was associated with almost four-fold increase in D-dimer concentrations. These results in agreement with previous published data showed that increased CRP and D-dimer upon admission in COVID-19 individuals is associated with the need to intensive care support and were strongly related to assisted ventilation[1, 2, 6, 9, 33]. A previous meta-analysis and meta-regression study examined the relationship between poorer outcomes and D-dimer values has reported high sensitivity but relatively low specificity in detecting COVID-19-related venous thromboembolism events[10]. Furthermore, the relationship between COVID-19 severity, death rate and D-dimer value was found to be restrained[10]. This is probably realistic since the reliance on more biomarkers gives a clearer image than the reliance on a single test in regards to disease prognosis. On the other hand, our results suggest that plasma ferritin together with CRP and D-dimer tests can be considered as first line tests when diagnosing and evaluating patients with COVID-19 upon admission.

In routine clinical practice, individuals with hyperferritinemia usually had multiple conditions, and the diagnosis of hyperferritinemia most often occurred incidentally as a result from laboratory screening or follow-up test[17]. Serologically, the significant increase in ferritin level or hyperferritinemia is difficult to interpret without the existence of observable clinical milieu. This is because hyperferritinemia is associated with a wide etiological spectrum and common concomitant existence of numerous etiologies ranged from malignancy to arthritis[34]. A previous study had reported that hyperferritinemia is a consequence of non-human immunodeficiency virus infection[17], while others found that malignancy is the most frequent cause of hyperferritinemia[34]. A part from its known role as an iron storage protein, ferritin can be undertaking other functions, since it might be actively secreted at the site of infection. Previous studies have considered ferritin as a signaling molecule and as an active contributor to the cytokine storm through it is pro-inflammatory effect and is consider as a key mediator of immune dysregulation directly through its suppressive effect especially under extreme hyperferritinemia[16, 18].

Hyperferritinemia in the current study was associated with a significant increase in WBC and IgM in COVID-19 patients presented with severe symptoms. Although, WBC has increased in COVID patients with mild symptoms, however this increase was insignificant and stayed within the normal range. Previous research showed that autoantibodies are directed to WBC and such antibodies may deplete T cells and B cells which are important to fight the virus[8]. In another word, significant decrease in leukocytes causes a state of immunodeficiency, weakening the body and doubling the risk associated with COVID-19. There was a significant increase in granulocytes in the current study and these results are in line with previously published data[7]. A previous study suggested that granulocytes can be used to identify COVID-19 severity[7]. Nevertheless, data regarding the roles of granulocytes in the pathogenesis of COVID-19 are scarce.

Hyperferritinemia in COVID-19 patients of the current study was associated with a significant reduction in lymphocytes, and our results are in agreement with previously published studies. Decreased lymphocytes or lymphopenia have been considered as one of the major serological characteristics of severe COVID-19 patients[3–5]. Clinically, lymphocyte's test is usually performed during routine medical encounters. Accumulative evidence showed that age plays an important role in the development lymphopenia. Thus, compared with children, lymphopenia is more common in elderly[35]. In line with these observations, recent studies reported a high incidence rate of lymphopenia in older COVID-19 patients compared with children[36]. A significant reduction in lymphocytes is known to play a crucial role in the pathogenesis of acute myocardial infarction and congestive heart failure[37, 38]. Thus, it is not surprising that lymphopenia in patients with severe COVID-19 is associated with decreased survival rate.

Finally, this study has several drawbacks, which include the reliance on one time point when collecting blood samples from patients with COVID-19. As a result, we could not have a proper image on the highest ferritin level in patients with severe symptoms in relation to infection progression nor we were able to detect the gradual decrease in ferritin levels and other studied biomarkers in relation to the treatment response and viral clearance. Second, we relied on relatively small sample size, which makes it not feasible to generalize our conclusion. Due to cultural and religious reasons, our previous studies[39, 40] have faced enormous challenges to collect the needed number of females, in contrast females have willingly joined the COVID-19 project and this can be considered among the strengths of the current study.

Conclusion

Hyperferritinemia is associated with poor prognosis in Arabs diagnosed with severe COVID-19. Severe symptoms of patients presented with COVID-19 included: difficulties in breathing, assisted ventilation, long stay in hospital, ICU admission, long COVID and decreased survival rate. In addition, the high ferritin level was found to be associated with hyper reaction of the immune system, resulting in a significant increase in serum CRP, WBC, IgM, granulocytes and D-dimer. In contrast, hyperferritinemia was associated with a significant reduction of lymphocytes. The results of the current study suggest that ferritin together with CRP and D-dimer concentrations can be considered as markers upon admission to predict disease severity and poor prognosis in Arabs diagnosed with COVID-19. These findings may limit the number of biochemistry

tests for individuals diagnosed with COVID-19, as they may provide a better image for patients who are in need for hospitalization and ICU admission. However, a larger cohort is needed in order to generalize this conclusion.

Acknowledgments
We would like to thank the clinicians, nurses and laboratory technologists in the Ramadi General Hospital for their assistance in this study. We also thank the Faculty of Science, University Of Anbar, Iraq, for technical support. Finally, we would like to express our gratitude to all the participants of this study.

Conflict of interest: none.

References

1. **Chen N., Zhou M., Dong X., et al.** Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395(10223), 507–513.
2. **Zhou F., Yu T., Du R., et al.** Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395, 1054–1062.
3. **Ghizlane E., Manal M., Abderrahim E., et al.** Lymphopenia in Covid-19: A single center retrospective study of 589 cases. Ann. Med. Surg. 2021; 69, 102816.
4. **Lee J., Park S. S., Kim Y. T., Lee D. G., Kim D. W.** Lymphopenia as a Biological predictor of outcomes in COVID-19 patients: A Nationwide Cohort Study. Cancers (Basel) 2021; 13(3), 471.
5. **Illg Z., Muller G., Mueller M., Nippert J., Allen B.** Analysis of absolute lymphocytes count in patients with COVID-19. Am. J. Emerg. Med. 2021; 46, 16–19.
6. **Petrilli C. M., Jones S. A., Yang J., et al.** Factors associated with hospitalization and critical illness among 4,103 patients with Covid-19 disease in New York City. MedRxiv 2020.
7. **Vitte J., Diallo A. B., Boumaza A., Lopez A., Michael M., Allardet-Servent J.** A granulocytes signature identifies COVID-19 and it is severity. J. Infect. Dis. 2020; 222(12), 1985–1996.
8. **Zhu B., Feng X., Jiang C., et al.** Correlation between white blood cell count at admission and mortality in COVID-19 patients: a retrospective study. BMC infect. Dis. 2021; 21, 574.
9. **Varikasuvu S. R., Varshney S., Dutt N., et al.** D-dimer, disease severity, and deaths (3D-study) in patients with COVID-19: a systematic review and meta-analysis of 100 studies. Sci. Rep. 2021; 11, 21888.
10. **Zhan H., Chen H., Liu C., et al.** Diagnostic value of D-dimer in COVID-19: A meta-analysis and meta-regression. Clin Appl Thromb. Hemost. 2021; 27, 1–10.
11. **Kernan K. F., Carcillo J. A.** Hyperferritinemia and inflammation. Int. Immunol. 2017; 29(9), 401–409.
12. **Ogilvie C., Fitzsimons K., Fitzsimons E.** Serum ferritin values in primary care: are high values overlooked? J. Clin. Pathol. 2020; 63, 1124–1126.
13. **Sandnes M., Ulvik R. J., Vorland M., Reikvam H.** Hyperferritinemia-A clinical overview. J. Clin. Med. 2021; 10(9), 2008.
14. **Beaton M. D., Adams P. C.** Treatment of hyperferritinemia. Ann. Hepatol. 2012; 11(3), 294–300.
15. **Carubbi F., Salvati L., Alunno A., et al.** ferritin is associated with the severity of lung involvement but not with worse prognosis in patients with COVID-19: data from two Italian COVID-19 units. Sci. Rep. 2021; 11, 4863.
16. **Rosario C., Zandman-Goddard G., Meyron-Holtz E. G., D'Cruz D. P., Shoenfeld Y.** The Hyperferritinemic Syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. BMC Med. 2013; 11(2013), 185.
17. **Senjo H., Higuchi T., Okada S., Takahashi O.** Hyperferritinemia: causes and significance in a general hospital. Hematology 2018; 23(10), 817–822.
18. **Abbaspour N., Hurrell R., Kelishadi R.** Review on iron and its importance for human health. Research J. Med. Sci. 2014; 19(2), 164–174.
19. **Abul Y., Leeder C., Gravenstein S.** Epidemiology and clinical presentation of COVID-19 in older adults. Infect. Dis. Clin. North Am. 2023; 37(1), 1–26.
20. **Wang D., Hu B., Hu C., et al.** Clinical characteristics of 138 hospitalized patients with 2019 novel Coronavirus-infected pneumonia in Wuhan, China. JAMA 2020; 323(11), 1061–1069.
21. **Nanda A., Vura N.V.R. K, Gravenstein S.** COVID-19 in older adults. Aging Clin. Exp. Res. 2020; 32, 1199–1202.
22. **Powell T., Bellin E., Ehrlich A. R.** Older adults and Covid-19: The most vulnerable, the hardest hit. Hastings Center Report 2020; 50(3), 61–63.
23. **Paget J., Spreeuwenberg P., Charu V., et al.** Global mortality associated with seasonal influenza epidemics: New burden estimates and predictors from the GLaMOR Project. J. Glob. Health 2019; 9(2), 020421.
24. **Feinkohl, I., Janke, J., Hadzidiakos, D., et al.** Associations of the metabolic syndrome and its components with cognitive impairment in older adults. BMC Geriatr. 2019; 19, 77.
25. **Assuncao N., Sudo F. K, Drummond C., de Felice F. G., Mattos P.** Metabolic syndrome and cognitive decline in the elderly: A systematic review. PLoS One 2018; 13(3), e0194990.
26. **Ali A. T., Al-ani O., Al-ani F.** Epidemiology and risk factors for ovarian cancer. Menopause Rev. 2023; 22(2), 93–104.
27. **Ali A. T.** Risk factors for endometrial cancer. Ces. Gynekol. 2013; 78(5), 448–459.
28. **Ali A. T.** Towards prevention of ovarian cancer. Current Cancer Drug Targets 2018; 18(6), 522–537.
29. **Ali A. T.** Can we prevent ovarian cancer? Ces. Gynekol. 2020; 85(1), 49–58.
30. **Ali A.T., Al-Ani F., Al-Ani O.** Childhood obesity: causes, consequences, and prevention. Čes. slov. Farm. 2023; 72(1), 21–36.
31. **Nour T. Y., ALTINTAŞ K. H.** Effect of the COVID-19 pandemic on obesity and its risk factors: a systematic review. BMC Public Health 2023; 23, 1018.

32. **Ho J. S. Y., Fernando D. I., Chan M. Y., Sia C. H.** Obesity in COVID-19: A Systematic review and meta-analysis. Ann. Acad. Med. Singap. 2020; 49(12), 996–1008.
33. **Dujardin R. W. G., Hilderink B. N., Haksteen W. E., et al.** Biomarkers for the prediction of venous thromboembolism in critically ill COVID-19 patients. Thromb. Res. 2020; 196, 308–312.
34. **Moore jr. C., Ormseth M., Fuchs H.** Causes and significance of markedly elevated serum ferritin levels in and academic medical center. J. Clin. Rheumatol. 2013; 19(6), 324–328.
35. **Acanfora D., Scicchitano P., Carone M., et al.** Relative lymphocyte count as an indicator of 3-year mortality in elderly people with severe COPD. BMC Pulm. Med. 2018; 18, 116.
36. **Tavakolpour S., Rakhshandehroo T., Wei E. X., Rashidian M.** Lymphopenia during the COVID-19 infection: What it shows and what can be learned. Immuno. Lett 2020; 225, 31–32.
37. **Acanfora D., Gheorghiade M., Trojano L., et al.** Relative lymphocyte count: a prognostic indicator of mortality in elderly patients with congestive heart failure. Am. Heart J. 2001; 142, 167–173.
38. **Nunez J., Sanchis J., Bodi V., et al.** Relationship between low lymphocyte count and major cardiac events in patients with acute chest pain, a non-diagnostic electrodiagram and normal tropnin levels. Atherosclerosis 2009; 206, 251–257.
39. **Al-Rawi K. F., Ali H. H., Guma M. A., et al.** Relationship between IL-2, IL-17 concentrations, and serum creatinine levels in men with chronic kidney diseases. Rep. Biochem. Mol. Biol. 2022; 10(4), 664–674.
40. **Ali H. H., Al-Rawi K., Khalaf Y., et al.** Serum caveolin-1 level is inversely associated with serum vaspin, visfatin, and HbA1c in newly diagnosed men with type-2 diabetes. Rep. Biochem. Mol. Biol. 2022; 11(2), 299–309.