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Changes in markers ferritin and C-reactive protein in patients with COVID-19 in Iraq: A case-control study

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***Abstract*---**Background: According to the coronavirus virus resource center of Johns Hopkins Medicine, more than 75 million people are presently affected worldwide, SARS-CoV-2 infect host cells through the angiotensin-converting enzyme 2 (ACE2) receptor, which is found in a variety of human organs, the virus's entry into the bloodstream has resulted in the involvement of practically all of the body's organs. Aim: We have aimed to determine the roles of serum ferritin and CRP in different strata of patients with COVID-19 infection and to predict disease severity. Methods: A retrospective study was carried out after obtaining approval from the relevant Ethics Committee. Total number of 125 cases taken for study, admitted on a priority basis. Consecutive blood tests that included ferritin and CRP in the study period were reviewed. Patients diagnosed with SARS-CoV-2 infection in whom,

serum ferritin and CRP had been analyzed at the time of admission were selected. For assessment of severity and behavior of the factors to be analyzed the COVID-19 patients were grouped into (mild),(moderate) and (severely ill) cases, factors analyzed in all groups and correlated,in December 27, 2020, to April 25, 2020. Results: For patients, the mean age was 51 years 64% were men. The comparison of some markers of inflammation between the patients with COVID-19 and the healthy controlled persons, the serum level of ferritin and CRP in patients with COVID-19 was higher than that in the controlled group, highly significant ($p < 0.001$). There was a highly significant difference in ferritin and CRP level among the patients according to the severity (($p < 0.01$); the level was higher in the severe cases.

***Keywords*---**COVID-19, ferritin, C-reactive protein.

Introduction

Since December 2019, a new type of coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), has been identified in China (Javanian *et al.*, 2020). The COVID-19 pandemic then spread quickly around the world (Hsiang *et al.*, 2020). Both 2019-nCoV and SARS-CoV enter the host cell via the same receptor, angiotensin-converting enzyme 2 (ACE2) (Zhou *et al.*, 2020). The clinical manifestations of COVID-19 disease are heterogeneous, including severe and non-severe forms. The management of patients is therefore adapted to the severity of the clinical situation. According to recent experiences, the majority of infected persons are not severely affected and can recover without medical intervention, whereas a small number of cases need to be carefully treated and hospitalized in an intensive unit (Huang *et al.*, 2020). Of this clinical parameter, serum C-reactive protein (CRP) has been found as an important marker that changes significantly in severe patients with COVID-19 (Wang *et al.*, 2020). The disease is not just confined to the lungs, but the spillage of the virus into the bloodstream has to lead to the involvement of almost all the organs of the body, including the heart, liver, brain, kidney, skin, intestines, and eyes (Singh *et al.*, 2021). CRP is a type of protein produced by the liver that serves as an early marker of infection and inflammation (Marnell *et al.*, 2005). In blood, the normal concentration of CRP is less than 10 mg/L; however, it rises rapidly within 6 to 8 hours and gives the highest peak in 48 hours from the disease onset (Young *et al.*, 1991). Its half-life is about 19 hours (Pepys and Hirschfield, 2003).

The synthesis of ferritin is regulated by various “oxidant and antioxidant stimuli” e.g. nitrous oxide, glutathione, and other “reactive oxygen species”. The ratio of expression of both FTH as well as FTL is influenced by the inflammatory process (Kernan and Carcillo, 2017). Iron regulatory proteins 1 & 2 (IRP1 or IRP2) alter the translation of FTH and FTL mRNA by interacting through a regulatory region on the mRNA which is known as the “iron response element” (IRE) in the “5′ untranslated region” (UTR) (Wang *et al.*, 2021).

There is mounting evidence that in critically ill patients, there are characteristics of hyper inflammation, which consist of elevated serum C-reactive protein (CRP), procalcitonin (PCT), D-dimer, and hyperferritinemia. These findings suggest a possibly crucial role of a cytokine storm in COVID-19 pathophysiology(Mehta *et al.*, 2020). CRP is an inflammatory marker and also an important factor that affects the development of inflammation. High serum levels of CRP are a result of an acute inflammatory condition; CRP measurement between 0.5 and 10.00 mg/L facilitates the detection of low-intensity chronic inflammatory processes (Khreiss *et al.*, 2004). During the clinical manifestation of Covid-19 infection interleukin-6 (IL-6) and CRP increase both drastically(G. Chen *et al.*, 2020). Hyperferritinemia caused by the excessive inflammation due to the infection is associated with admission to the intensive care unit and high mortality and represents an indication to recognize high-risk patients to guide the therapeutic intervention to control inflammation (Carcillo *et al.*, 2017).

Materials and Methods

Study Design, Participants, and Definition

After approval obtained from Al-Qadisiyyah University Ethics Committee (Grant number:) and Ministry of Health this study was initiated with a retrospective design that enrolled patients hospitalized with COVID-19 at AL-Diwaniya Teaching Hospital. A total of 65 persons were confirmed at these center from December 27, 2020, to April 25, 2020. All patients with COVID-19 who enrolled in the recent study were diagnosed according to, all patients with the physician- and laboratory-confirmed (positive nasopharyngeal/throat swab specimens by reverse transcription-polymerase chain reaction (RT-PCR)) COVID-19 infection were included, while suspected cases with similar clinical symptoms Patients aged below 18, pregnant women, and individuals with cancer were excluded. One of the following criteria was used to determine severe COVID-19 illness: respiratory rate ≥30 bpm, oxygen saturation ≤93%, arterial oxygen partial pressure (PaO_2)/oxygen concentration (FiO_2) ≤300 mm Hg, and intensive care unit (ICU) admission.

Study Population

The study included 125, both males and females, ranging in age from 18 to 82 years, 65 COVID-19 patients attending Al-Diwaniyah Teaching Hospital in Iraq, and 60 healthy controls. In the healthy controls, there were 31 men and 29 women, whereas, in COVID-19, there were 42 men and 23 women.

Evaluation Biomarkers (CRP and Ferritin) of Patients With COVID-19

All the cases were labeled as mild, moderate, and severe based on the clinical symptoms, laboratory tests, and chest computed tomography (CT) scans. Moderate and severe cases were classified as having pneumonia manifestations seen on imaging and oxygen saturation ≤ 90% at rest. severe cases were labeled as those requiring mechanical ventilation due to respiratory failure, other organ failures that require intensive care unit. The control group consists of (60) uninfected people who have not previously shown symptoms of coronavirus, the

diagnosis, and selection of the patient are carried out by the doctor present in the isolation unit at Al-Diwaniyah Teaching Hospital. done via electrochemiluminescent immunoassay (ECLIA) in the Elecsys immunoassay system. All the parameters were analyzed and compared between the Covid-19 and the control group. (CRP, reference range: ≤ 5 mg/L), (REFERENCE VALUES Men: 125– 220 U/L. Women: 20 – 110 µg/L).An increase in deviation from normal values may be at risk for infection COVID-19.

Statistical Analysis

Statistical analysis between allele frequencies and genotype distributions for the four groups was confirmed by descriptive statistics using SPSS 23 software by mathematical at (p-value ≤ 0.001). The comparison between groups for biochemical and immunohistochemical tests was performed by means, of a double sample, at (p-value ≤ 0.001) as it was a significant difference. The statistical analysis between the biomarkers (CRP, ferritin) in the two groups (Control group, and patients with COID-19 group) was confirmed by the descriptive statistics at (p-value ≤ 0.05).

Results and Discussion

The study population included 65 hospitalized patients with COVID-19, in which 16 cases were mild, 23 cases moderate and 21 cases were severe, They were diagnosed by the doctor based on the patient's condition, computerized tomography scanning the blood test, and the degree saturation of oxygen, For the healthy persons were 60, severe and moderate cases patients with covid-19. On admission, most patients had fever, cough, mild to moderate dyspnea, myalgia, chest discomfort, Severe patients had a (%SpO_2) of less than 40%.

Table 1. Comparison of markers of inflammation between patients with COVID-19 and healthy control subjects

Characteristic	**Control group *n* = 60**	**COVID-19 group *n* = 65**	***p***
CRP (mg/L)			
Median (IQR)	2.30 (2.39)	87.00 (120.73)	< 0.001 M HS
Range	0.37 -9.1	4.14 -405	
Ferritin			
Median (IQR)	35.00 (36.58)	401.00 (369.44)	< 0.001 M HS
Range	11.5 -200.59	96.2 -1351	

The results of the present study showed a significant increase that was observed in the CRP is levels in patients with SARS-CoV-2 as compared with healthy individuals 87.00(120.73),2.30(2.39) respectively and the difference was highly significant (p < 0.001) as it is explained in Table 1.These results corresponded with the previous study (Yufei *et al.*, 2020). In this study increased serum levels of ferritin in patients with COVID-19 which was higher than in the controlled group, 401.00(369.44) versus 35.00(36.58) Table 1.

Table 2. Comparison of inflammatory markers according to the severity of disease

Characteristic	Mild n = 16	Moderate n = 28	Severe n = 21	p
CRP	48.27 (49.79)	74.25 (72.98)	162.28 (123.10)	0.001 K HS
Ferritin	314.60 (454.37)	337.00 (256.25)	550.00 (470.42)	0.007 K HS

The CRP concentration increased significantly ($p<0.05$) in the severe group 162.28(123.10) mg/L as compared with the mild and the moderate cases 48.27(49.79), 74.25(72.98) mg/L respectively, which was consistent with the findings of a previous study in the chain (W. Chen *et al.*, 2020). These results correspond with the studies (Shi *et al.*, 2020) (H. Zhang *et al.*, 2020)(G. Zhang *et al.*, 2020).and the consequences in the present study are matched with (W. Chen *et al.*, 2020; Yufei *et al.*, 2020).

The elevated levels of CRP might be linked to the overproduction of inflammatory cytokines in severe patients with COVID-19. CRP production is induced by inflammatory cytokines and by tissue destruction in patients with COVID-19. In conclusion, an elevated level of CRP may be a valuable early marker in predicting the possibility of disease progression in non-severe patients with COVID-19 (Ali, 2020).

There was a highly significant difference in ferritin level among patients according to severity (p = 0.007); the level was high in severe cases as explained in **Table 2**. These results correspond with the studies (Taneri *et al.*, 2020)(Cheng *et al.*, 2020; Abobaker, 2021; Deng *et al.*, 2021). Previous research studies have revealed the role of hyperferritinemia in assessing the severity of disease among COVID-19 patients (Wessling-Resnick, 2018; Lalueza *et al.*, 2020). and the difference was highly significant ($p<0.01$), These results are according to (Lino *et al.*, 2021).
The possible mechanisms include increased ferritin synthesis by proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and IL-lβ. This leads to increased inflammation that causes cell damage and the release of ferritin (Wessling-Resnick, 2018). Hyperferritinemia patients were found to have increased mortality risk; which is not clear whether it is a coincidence or due to viral pathogenesis (Lalueza *et al.*, 2020).

Increased levels of ferritin due to cytokine storm and Shih have also been reported in severe COVID-19 patients. During the cytokine storm in COVID-19, many inflammatory cytokines are rapidly produced, including IL-6, TNF-α, IL-1β, IL-12, and IFN-γ, which stimulate hepatocytes, Kupffer cells, and macrophages to secrete ferritin. The uncontrolled and dysfunctional immune response associated with macrophage activation, hyperferritinemia syndrome. Notably, ferritin is not only the result of excessive inflammation, but it also plays a pathogenic role in the inflammation process through its bind with the T-cell immunoglobulin and mucin domain 2 (TIM-2) by promoting the expression of multiple pro-inflammatory mediators (Cheng *et al.*, 2020).

Previous studies have shown that SARS and MERS can both cause liver dysfunction and current observations show that SARS-CoV-2 can similarly cause liver injury. Liver enzyme elevations in the setting of COVID-19 have been reported in cohort studies, occurring more often in patients with severe disease. Patients display a hepatocellular pattern of injury with elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Da *et al.*, 2020).

That COVID-19 can trigger an immune-mediated liver injury in certain individuals who develop severe liver dysfunction which is related to an exaggerated cytokine storm and acute respiratory. Histological examination, evidence of over-activation of T cells and increased Th17 and high cytotoxicity of CD8 T cells has been reported. Another possible mechanism. Injury via an ischemic process related to hypoxia. Reports from China suggest a higher rate of AST compared to ALT elevation consistent with ischemic liver injury due to hepatic zone coagulative necrosis. It was also significantly elevated LDH levels in all patients (Da *et al.*, 2020). The increased levels of ferritin could be the role of iron metabolism in supporting the innate immune system to fight invading microorganisms. The innate immune system orchestrates control over iron metabolism as a response to viral infections (Wessling-Resnick, 2018).

Therefore, the innate immune system will react by decreasing the bioavailability of iron to limit the replication of the virus during acute infection. Through interleukin-6 and Toll-like-receptor-4 dependent pathways, the levels of the liver-derived iron hormone hepcidin-the master regulator of iron homeostasis- could increase and block, the activity of the transporter ferroportin which carries iron out of the cells, and therefore decrease the amount of iron absorbed from the diet, causing cellular sequestration of iron (i.e., principally in hepatocytes, enterocytes, and macrophages). Increased intracellular iron sequestration will lead to an upregulation of cytosolic ferritin, which sequesters and stores iron to prevent iron-mediated free radical damage. The increased retention and storage of iron within ferritin in macrophages contribute to the characteristic fall in serum iron concentrations and an increase in serum ferritin concentrations as it was observed in an acute phase response (Taneri *et al.*, 2020). Increased levels of ferritin due to cytokine storm and secondary hem phagocytic lymphohistiocytosis were found in severe COVID-19 patients (Cheng *et al.*, 2020).

Table 3. The characteristics of receiver operator characteristics (ROC) curve analysis for CRP and ferritin

characteristic	**CRP**	**Ferritin**
Cutoff	0.9	0.9
AUC	0.997	0.985
95CI%	0.991 to 1.00	0.970 to 1.000
P	< 0.001 HS	< 0.001 HS
Sensitivity	0.985	0.969
specificity	1.000	0.933
Accuracy	99.70%	98.50%

The characteristics of the receiver operator characteristics (ROC) curve analysis for CRP and ferritin are shown in table 3. and figure 1. It appears that CRP is more sensitive and more specific, from ferritin

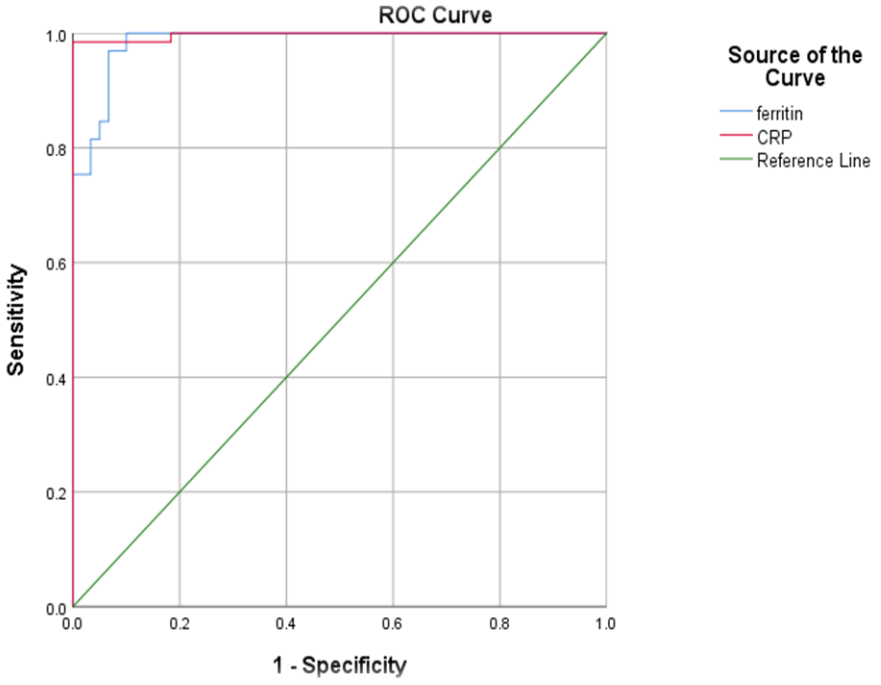

Figure1.The receiver operator characteristic (ROC) curve analysis for CRP and ferritin cutoff values in the diagnosis of COVID-19.

To further assess the predicting power of the two diagnostic factors mentioned above, ROC curve analysis was used. Although CRP showed higher sensitivity (0.985) in the ROC curve, the area under the curve (AUC) (0.997), However, ferritin exhibited the AUC of (0.985). The results revealed that CRP had high predicting power than that of ferritin for COVID-19 patients. COVID-19 patients with increased CRP or increased ferritin level may be at risk for COVID-19 infection, which indicates that it is useful for CRP and ferritin to predict COVID-19 patients.

Conclusion

This research found an association between serum ferritin levels and C-reactive protein levels in COVID-19 patients, as well as an important link between elevated ferritin and CRP levels and COVID-19 severity. The results can be used as a pre-epidemic biomarker to improve the management of COVID-19 patients by identifying infected individuals and using appropriate treatment**.**

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