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Evaluation of some laboratory parameters and their diagnostic and prognostic role in COVID-19 patients admitted to Syrian hospitals

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ABSTRACT

A serious threat to global health is posed by COVID-19, particularly to the health system in Syria, which has been decimated by the protracted war. COVID-19 in Syria has resulted in a significant number of cases that need hospitalization and intensive monitoring. COVID-19 causes a multisystem failure of many organs, mainly, heart disease. Therefore, our study aims to evaluate the role of inflammatory laboratory parameters and cardiac enzymes of COVID-19 patients at several phases of follow-up in order to evaluate their importance in deciding whether patients will be remained to the hospital or discharged. The study included the measurement Ferritin, CRP, CK-MB and AST levels in 45 COVID-19 patients in two hospitals in Syria and 30 volunteers as a control group. This study found that odds ratio (OR) for CRP was the highest among the studied indicators. Ferritin levels were different between the surviving and deceased patients in the admission sample, and there was a significant difference between ferritin and CRP at the admission and discharge samples for the patients who were still alive. Interestingly, 42.2% of patients had elevated CK-MB and AST levels that were higher than normal values. The most crucial criterion for the diagnosis of COVID-19 infection was CRP, while ferritin was the most significant signal for the prognosis of death.

1. Introduction

Coronaviruses (COVs) spread among humans and usually cause mild respiratory diseases. Some of these coronaviruses led to significant pandemics that killed a lot of infected people and caused severe respiratory illnesses in infected individuals. Of these pandemics; the severe acute respiratory syndrome pandemic due to the SARS-COV in 2002 and the Middle East respiratory syndrome due to the MERS-COV virus in 2012 [1,2]. In January 2020 the world health organization (WHO) declared a state of public health emergency as result of the outbreak of Sever Acute respiratory syndrome coronavirus 2 (SARS-COV-2) that caused the coronavirus disease (COVID-19) in many countries of the world [3].

Coronaviruses seem to appear periodically in humans due to the high prevalence, large genetic variety and recurrent recombination of their genomes. Along with an increase in activities involving animal-human interaction [4,5].

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COVID-19 causes a high inflammatory response which might lead to a multisystem failure of many organs with a high mortality rate [6,7]. The severe inflammatory response brought on by COVID-19 is accompanied by the cytokine storm (CS), a process where a significant number of inflammatory cytokines are released [8]. Many immune cells, such as the adaptive B and T lymphocytes, natural killer cells, innate macrophages, and dendritic cells, release cytokines, which are crucial to the inflammatory response [9]. Many studies on COVID-19 patients have revealed a connection between the cytokine storm and the severity of the infection and the probability of death [8,10]. The inflammatory process is featured by many changes in the level of certain proteins in the plasma. These proteins are known as the acute phase proteins (APPs) which are part of the many systemic symptoms of acute-phase response. Ferritin and c-reactive protein (CRP) are the most popular APPs used as markers in clinical practice [11,12].

COVID-19 patients have also functional abnormalities of multi organs and systems, e.g., hematological, cardiovascular, in addition to the respiratory compromise, which may develop into acute respiratory distress syndrome (ARDS) [13]. As the systemic inflammation, the expression of virus receptors ACE2, and the direct entry of the virus into the cell are all factors that lead to the damage to systems, as many studies have demonstrated that these receptors are expressed on the surface of heart cells [14]. Consequently, Evaluation of cardiac enzymes levels (CK-MB, AST) in COVID-19 patients also prompted the study to their clinical significance.

The first COVID-19 case was announced in Syria on 22 March 2022, and the first death due to COVID-19 was announced a week later [15,16]. In a 2020 WHO report about Syria, it was confirmed that people who have chronic diseases including (heart diseases - diabetes mellitus) are more likely to be seriously infected and death due to Covid-19. This risk is exacerbated by the interruption of adequate preventive services. The World Health Organization has recommended early diagnosis, treatment and rehabilitation of the COVID-19 patients in Syria [17]. Consequently, our study soughted to evaluate the role of inflammatory laboratory parameters and cardiac enzymes in patients with COVID-19 in the Syrian government hospitals. This is the first study in Syria that aims to analyze of some laboratory results of COVID-19 patients at several phases of follow-up in order to assess their significance in determining whether patients would be discharged from the hospital or remain there, find cut-off values for these parameters that could be diagnosis of disease and predictive of death caused. In addition, investigate the relationship between the examined laboratory parameters and the long-COVID syndrome.

2. Materials and Methods

2.1 Study Participants

2.1.1 Patients group and study design

This prospective, multicenter, observational study included 45 patients, 28 (62.2%) males and 17 (37.8 %) females aged (34-85) with an average of 62.4 years. Which was conducted at two hospitals in Aleppo - Syria: Al-Razi Hospital and University Cardiac Surgery between November 1, 2021 and March 16, 2022. Patients provided personal consent to conduct the research and their consent to conduct further laboratory procedures after their discharge from the hospital. Our study was approved by directorate of communicable and chronic diseases, ministry of health, Syria, and was approved by Ethics Committee at Faculty of Pharmacy in Aleppo University.

2.1.2 Inclusion criteria

A total patients met 45 patients the inclusion criterion, adult patients admitted to the hospital and confirmed to be infected with COVID-19 by the laboratories of the Syrian Ministry of Health through the real-time polymerase chain reaction assay (RT-PCR), and we also allowed admission of patients with no prior medical history and patients with chronic diseases that did not interfere with the studied laboratory parameters (e.g., diabetes mellitus type 2 (DM2), hypertension (HTN), hyperlipidemia).

2.2.3 Exclusion criteria

Exclusion criteria were: all cases affecting the values of the investigated laboratory parameters were excluded, hemolyzed specimens, patients with hepatic disease, heart disease (e.g., heart failure, ischemic heart), cancer patients, autoimmune disease patients, patients who were admitted to the hospital before they contracted COVID-19, as well as patients who arrived from other hospitals more than 24 hours after the diagnosis of the illness.

2.1.4 Diagnosis of SARS-CoV-2 infection

It relied on the laboratories of the Syrian Ministry of Health to diagnose SARS-CoV-2 infection. Briefly, viral RNA was isolated from nasopharyngeal swabs or tracheal aspirates from subjects with suspected COVID-19 using The PhoenixDx SARS-CoV-2 Multiplex (Catalog no. 15262, Procomcure Biotech GmbH, Thalgau, Austria), using Biorad Miniopticon Real Time PCR analyzer. The PhoenixDx SARS-CoV-2 Multiplex test is based on conventional RT-PCR technology including extraction and purification of the nucleic acid genome of SARS-CoV-2 from the patient sample followed by PCR amplification and detection.

Nucleic acid from patient samples and controls are extracted in parallel using the RTA Viral Nucleic Acid Isolation Kit. Nucleic acid is released by the lysis reagent and bound to the silica columns. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted silica columns with elution buffer. External controls (positive and negative) are processed in the same way with each run.

Finally, Selective amplification of target nucleic acid from the sample is achieved by the use of targetspecific forward and reverse primers and probes specific to conserved regions of the ORF1ab and N genes for SARS-CoV-2.

2.1.5 Control group

The control group included 30 volunteers of healthy people from the community; 15 (50%) males and 15 (50%) females aged (31-73) years with an average of 50.3 years.

2.2. Blood sampling

Blood samples were collected into two blood collection tube groups during the first 24 hours of patient's admission to the hospital, contained lithium heparin anticoagulant while the other contained EDTA. Samples were then centrifuged; the plasma was collected in Eppendorf tubes and frozen at -15 °C. In addition, peripheral blood samples were collected from the surviving patients on the day they were discharged from the hospital. Further peripheral blood samples were collected from surviving patients two and four weeks after their discharge. A continuous follow up of patients was carried out until all clinical symptoms were absent and the duration of each symptom was recorded. Samples were collected and analyzed according to the centers for disease control and prevention (CDC) guidelines [18].

2.3. Testing methods of the evaluated biomarkers

Ferritin, CRP, CK-MB, AST and were assayed during the first 24 hours in the central laboratory of Aleppo University Hospital using BioSystems BTS-350 analyzer (BioSystems, Spain) based on the spectrophotometer technique. The following BioSystems kits were used: Ferritin Turbidimetry Kit, CK-MB Immune Inhibition Kit, CRP Kit and AST IFCC Kit. And complete blood count (CBC) was assayed using Genrui KT-6400 analyzer.

2.4. Statistical analysis

Statistical tests were carried out using statistical product and service solutions (SPSS) version 26 (BM Corp., NY, USA). This included the two independent samples T-test and Mann-Whitney U, Wilcoxon, in addition to ROC curve and logistic regression analysis, and in a case of nominal variables, Phi and Cramer test were used. The values of $P<0.05$ were considered statistically significant.

3. Results

3.1. Demographic and Clinical characteristics and Comorbidities in patient's group:

Upon admission to the hospital, the most common symptoms for patients were dyspnea 39/45 (86.7%), fever 37/45 (82.2%), cough 34/45 (75.6%), and general fatigue 33/45 (73.3%). Among patients the most common comorbidities were HTN 22/45 (48.9%), DM2 16/45 (35.6%), and chronic kidney disease (CKD) 3/45 (6.7%).

The total number of deaths among the patient's group was 23 (51.1%) deaths with 19 (42.2%) patients died during hospitalization and 4 patients died during less than two weeks after discharge of hospital. Table 1 shows Patients' Clinical characteristics and compares the two groups (survival and mortality). There were statistical differences in smoking (P = 0.048), and dyspnea (P = 0.036).

Table 1. *Data of COVID-19 patients: comparison of two groups (survival and death).*

DATA	Total No. of patients N=61	Survival N=26	Death N=35	P-values
Age, mean ± SD.	63.93 ± 11.97	60.3 ± 11.8	66.6 ± 11.5	**0.043**
Sex, Male (% of Male).	43 (70.5%)	16 (61.5%)	27 (77.1%)	0.186
Vaccine, yes (% of yes)	6 (9.8%)	5(19.2%)	1(2.8%)	**0.034**
Smoking.	19 (31.1%)	12 (46.2%)	7 (20%)	**0.029**
HTN*.	30 (49.2%)	14 (53.8%)	16 (45.7%)	0.530
DM2**.	25 (41%)	12 (46.2%)	13 (37.1%)	0.479
Hyperlipidemia.	3 (4.9%)	2 (7.7%)	1 (2.9%)	0.388
Renal failure.	4 (6.6%)	1 (3.8%)	3 (8.6%)	0.461
Asthma.	3 (4.9%)	2 (7.7%)	1 (2.9%)	0.388
Dyspnea.	54 (88.5%)	20 (76.9%)	34 (97.1%)	**0.001**
Fever.	47 (77%)	19 (73.1%)	28 (80%)	0.525
Cough.	43 (70.5%)	17 (65.4%)	26 (74.3%)	0.751
General fatigue	45 (73.8%)	21 (80.8%)	24 (68.6%)	0.284

* HTN: Hypertension, ** DM2: Diabetes mellitus type 2.

3.2. Diagnostic parameters of SARS-COV-2 infection

The comparison of studied parameters showed an elevation in the levels of ferritin, CRP, creatine kinase-MB (CK-MB), aspartate aminotransferase (AST) level in COVID-19 patients (927.4 µg/L, 73.8 mg/L, 37.7 U/L, 51.4 U/L, respectively) compared to the control group (107.4 µg/L, 4.1 mg/L, 12.8 U/L, 26.2 U/L, respectively). This difference was found to be statistically significant for all studied laboratory parameters (P-values < 0.001). Table 2 shows the significance of the studied parameters.

Table 2. *Comparison of laboratory parameter values between patient and control sample*

Laboratory parameters	Patient (N=61)	Control (N=30)	P-values
Ferritin (µg/L)	1003.9	107.4	**<0.001**
CRP (mg/L)	79.6	4.1	**<0.001**
CK-MB (U/L)	36.1	12.8	**<0.001**
AST (U/L)	49.5	26.2	**<0.001**

To further test the relationship between each evaluated parameters and diagnoses of COVID-19, receiver operating characteristic (ROC) curve was constructed and values of area under the curve (AUC) were found to be greater than 0.8 (0.996, 0.920, 0.858, 0.837; respectively) using cut-offs of 8.5 mg/L for CRP, 230.35 µg/L for ferritin, 17.5 U/L for CK-MB, and 29.5 U/L for AST, with sensitivity was (1, 0.889, 0.8, and 0.778; respectively) and specificity was (0.967, 0.967, 0.833, and 0.767; respectively). Figure 1 shows the ROC curve for studied parameters.

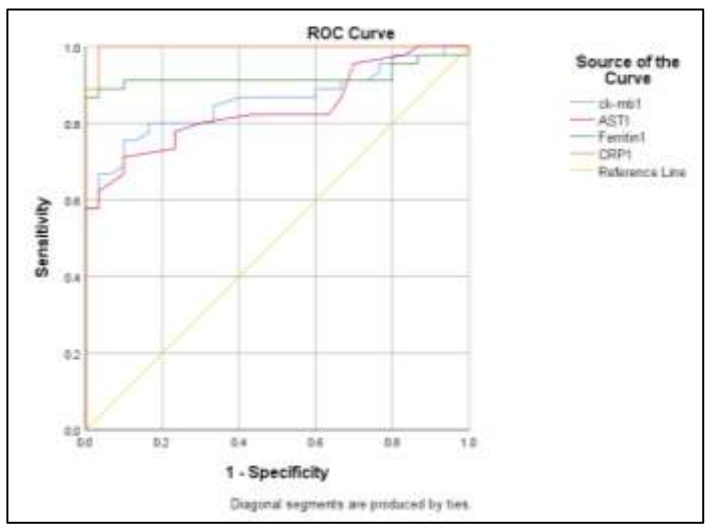

Figure 1: *ROC curve for laboratory parameters to diagnosis of COVID-19.*

Depending on the logistic regression model, The odds ratio (OR) for diagnosis was for each of the studied parameters, AST, CK-MB and ferritin, was (1.799, 1.156, 1.159, 1.013; respectively), CRP (OR: 1.799; 95% CI: 1.195-2.708; p = 0.005), CK-MB (OR: 1.159; 95% CI: 1.072-1.254; p < 0.001), AST (OR: 1.156; 95% CI: 1.070-1.250; p < 0.001), ferritin (OR: 1.013; 95% CI: 1.006-1.021; p < 0.001).

3.3. Laboratory tests in the hospital

The comparison of the values of laboratory parameters between survival and death groups of the admission sample, the results showed a significant increase in ferritin levels, WBCs number, RBCs number, neutrophil number, lymphocyte number and neutrophil lymphocyte ratio (NLR) for survival group (619.3 µg/L, 7.9×10^3/µl, 4.5×10^6/µl, 6.4×10^3/µl, 1.1×10^3/µl and 6.6; respectively) compared to the levels of these parameters for death group (1222.1 µg/L, 12.3×10^3/µl, 4.1×10^6/µl, 10.9×10^3/µl, 0.8 $\times10^3$/µl and 16.9; respectively), while the other parameters had no statistical significance. The results of the comparison are shown in Table 3.

***Table 3**. Laboratory parameters of 61 COVID-19 patients: Comparison of two groups (survival and death).*

Laboratory parameters	All patient N=61	Survival N=26	Death N=35	P-values
Ferritin (µg/L)	1003.9	641.9	1272.8	**0.004**
CRP (mg/L)	79.6	74.5	83.4	0.479
CK-MB (U/L)	36.1	37.5	35	0.699
AST (U/L)	49.5	46.6	51.6	0.550
WBCs ($\times10^3$/µl)	10	7.9	11.6	**0.01**
RBCs ($\times10^6$/µl)	4.2	4.3	4.2	0.325
Hemoglobin (g/dl)	12.1	12	12.1	0.422
Neutrophil ($\times10^3$/µl)	8.6	6.5	10.1	**0.008**
Lymphocyte ($\times10^3$/µl)	0.9	1.1	0.8	**0.026**
NLR	12.5	7.7	16	**0.001**

Furthermore, it was found that the levels of ferritin, CRP and CK-MB was significant higher, in surviving patients at admission to the hospital (619.3 µg/L, 66.9 mg/L, 37.8 U/L; respectively) compared to their levels when patients were discharged (393.7 µg/L, 13.4 mg/L, 23.8 U/L; respectively). Table 4, shows a comparison of the laboratory parameters of patients at admission to the hospital and at the time of discharge.

It was also found that 42.2% (19/45) of patients had an increased value both of CK-MB (above the upper limit of its value in the control group, which was 27.6U/L), and AST (above the upper limit of its value in the control group, which was 40U/L). Upon reviewing the patients' data, it was found that 24.4% (11/45) of the patients had a history of previous chronic diseases (hypertension – hyperlipidemia) While 17.7% (8/45) of those patients had no prior chronic diseases (HTN – hyperlipidemia) diseases.

***Table 4.** Laboratory parameters of surviving patients at admission and at the time of discharge.*

Laboratory parameters	Admission sample N=26	Discharge sample N=26	P-values
Ferritin (µg/L)	641.9	394.2	**0.007**
CRP (mg/L)	74.5	18.5	**0.001<**
CK-MB (U/L)	37.5	25.1	**0.003**
AST (U/L)	46.6	62.7	0.476
WBCs ($\times10^3$/µl)	7.9	8.6	0.125
RBCs ($\times10^6$/µl)	4.3	4.4	0.223
Hemoglobin (g/dl)	12	12	0.509
Neutrophil ($\times10^3$/µl)	6.5	6.8	0.354
Lymphocyte ($\times10^3$/µl)	1.1	1.3	0.144
NLR	7.7	7.2	0.620

In addition, when following up the patients, we have reported the death 4 of patients within less than two weeks after their discharge from the hospital. It was interesting to observe a significant difference in the ferritin values between those patients when discharge (1256 µg/L) and the surviving patients throughout the follow-up period (393.7 µg/L).

3.4. Biomarkers to predict death

ROC curves analysis was carried out to test the relationship between the evaluated parameters and mortality. It was found that only ferritin and NLR presented a significant value less than 0.05 (0.033, and 0.001; respectively) using cut-offs of 709.7 µg/L for ferritin, and 8.1 for NLR. Using these cut-offs, it was observed that AUC values are (0.686, and 0.77; respectively), with sensitivity was (0.609, and 0.696; respectively) and specificity was (0.682, and 0.864; respectively). Figure 2 shows ROC curve for ferritin, and NLR. Depending on the logistic regression model, ferritin ≥ 709.7 µg/L (OR: 1.001; 95% CI: 1.00-1.002; p = 0.042) and NLR ≥ 8.1 (OR: 1.21; 95% CI: 1.026-1.427; p = 0.023) continued to be independently linked to mortality.

3.5. Long-COVID syndrome

3.5.1. Symptoms Persistence

It was found that general fatigue was the most common symptom for patients, with a median of 29.5 days since the onset of symptoms until recovery (maximum 107 days). Also, the cough persisted for a median of 17 days (with a maximum of 89 days) from the onset of symptoms to the clinical absence of symptoms, as shown in Table 5.

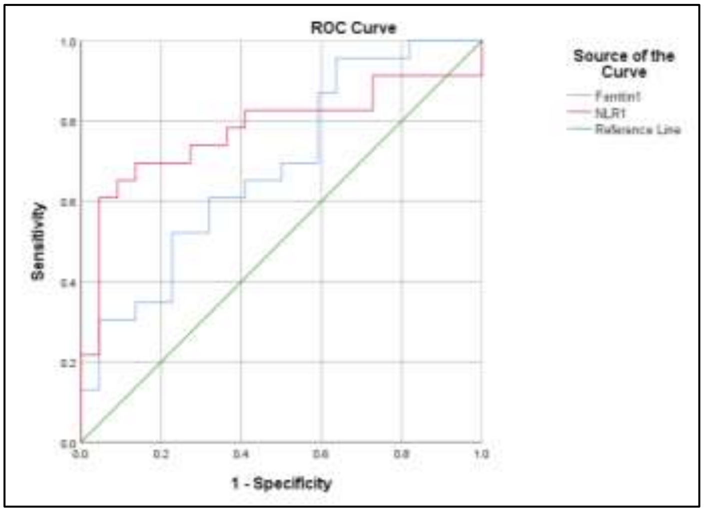

Figure 2. *ROC curve for parameters that can predict mortality.*

Table 5. *Average and maximum of stay the symptoms for patients.*

Symptoms	**Ave. (day)**	**Max. (day)**
general fatigue	36.1	107
cough	25.5	89
Loss of appetite	22	61
Dyspnea	21.5	47
Hoarseness	23.3	31
loss of sense of taste	23.7	28
loss of sense of smell	18.4	28

3.5.2. Correlation of laboratory parameters with persistence of symptoms

When evaluating the levels of previous laboratory parameters two weeks after the patients discharge from the hospital, it was noticed that the patient's ferritin levels were associated with the number of days of persistence of general fatigue and cough. Regarding the laboratory values' follow-up one month after discharge, only a correlation between ferritin levels and fatigue persistence was found; no correlation was found between any other laboratory parameters and the persistence of symptoms (Table 6).

Table 6. *The correlation of persistent general fatigue and cough with serum ferritin and CRP levels.*

		Pearson correlation coefficient	**P-values**
Two weeks after discharge from the hospital	General fatigue and serum ferritin levels	0.515	**0.020**
	General fatigue and CRP levels	0.495	**0.027**
	Cough and serum ferritin levels	0.797	**0.001<**
	Cough and CRP levels	0.187	0.430
A month after being discharged from the hospital	General fatigue and serum ferritin levels	0.729	**0.001**
	General fatigue and CRP levels	0.461	0.072
	Cough and serum ferritin levels	0.012	0.966
	Cough and CRP levels	0.137-	0.614

3.5.3. Lab abnormalities after discharge from the hospital

Laboratory parameters of the patients who were discharged from the hospital during a period of 15 to 20 days after discharge were examined. These parameter values were compared with the control sample to verify that the patients restored the normal values of the studied laboratory parameters.

The results showed that the values of ferritin and CRP for patients after discharge from the hospital remained high (269. 6µg/L and 11.4 mg/L; respectively) compared to the control group (107.417 µg/L and 4.1 mg/L; respectively) as illustrated in Table 7.

Table 7. *Comparison of the laboratory parameters for control sample with the patients after two weeks of discharge.*

Laboratory parameters	**Control (N=30)**	**Patients (N=21)**	**P-values**
Ferritin (µg/L)	107.4	273.4	**0.001<**
CRP (mg/L)	4.1	13.1	**0.002**
CK-MB (U/L)	12.8	18.9	0.078
AST (U/L)	26.2	29.5	0.075

Patients were contacted for the second time after discharge from the hospital within 30 to 35 days, and their laboratory parameters were assessed. When comparing the results of the laboratory values of patients with the results of laboratory values in control group, a significant difference was noticed for ferritin only (149.9 µg/L) for patients and (107.4 µg/L) for control as illustrated in Table 8.

Table 8. *Comparison of the laboratory parameters for control sample with the patients after a month of discharge.*

Laboratory parameters	Control (N=30)	Patients (N=16)	P-values
Ferritin (µg/L)	107.4	191.7	**0.009**
CRP (mg/L)	4.1	11.4	0.917
CK-MB (U/L)	12.8	15.5	0.128
AST (U/L)	26.2	27.18	0.556

4. Discussion

In this study, ferritin, CRP, CK-MB and AST were measured in COVID-19 patients and a control group of healthy volunteers. Statistical analysis using logistic regression supported the findings that these indicators have a significant diagnostic benefit since they were higher in the patient group. The results of this investigation shown that, although ferritin was a significant predictive indicator, CRP played a superior impact in predicting the diagnosis of COVID-19. The odds ratio (OR) and AUC of CRP was higher than ferritin when applying the logistic regression test and ROC curve, which indicates that CRP has a greater role in predicting the diagnosis of infection.

Multiple studies found a difference between the values of laboratory parameters on COVID-19 patients with positive RT-PCR and other hospital patients with negative RT-PCR for SARS-COV-2, in order to determine cut-off values that can be trusted to determine whether a patient admitted to the hospital has COVID-19 infection or not. [19,20]. The control sample in our study was collected from healthy people in the community in order to determine the cut-off values of the studied parameters, which we can rely on to confirm the infection of asymptomatic people who were in contact with patients who had COVID-19 and those who were accompanying patients in hospitals. The control sample in a Chinese study also was collected from healthy people, and the cut-off levels for ferritin and CRP were (109.8 g/L and 10.15 mg/L, respectively) [21]. We noted that the cut-off values for CRP are close to it in our study (8.5 mg/L) in contrast to ferritin (230 µg/L). This could be explained by the fact that hospitalized patients in our study and the Chinese study had significantly different average ages (62.4 and 49 years, respectively), as ferritin levels can rise with aging under the influence of the chronic, silent inflammatory disease. [22].

Our findings revealed a relatively high hospital mortality rate of 51.1%, even when compared to other countries. For example, a multicenter study in Sudan reported that 21% of COVID-19 patients died in hospitals [23]. Another study from Lebanon, showed a hospital mortality rate in COVID-19 patients of 19% [24]. For high-income countries, a study of COVID-19 patients in England, revealed a hospital mortality rate of 25% [25]. However, a different study conducted in Syria found that the fatality rate in Lattakia - Syria, was also great, at 48% [26]. The effect of the crisis on the healthcare system, as well as a lack of human resources, medications, and vaccination (8.9% in our study), are potential reasons for the high mortality rate in Syrian healthcare institutions. It caused the general public's health to decline and COVID-19 patient mortality rates to rise.

Ferritin levels and NLR in the admission sample were considerably different between the two groups of survivors and fatalities, according to the findings of our study, and as a result, they serve as useful prognostic indicators for estimating the probability of death.

The findings demonstrated that, in comparison to the four patients who passed away less than two weeks after leaving the hospital and the patients who lived through the entire follow-up period, the ferritin levels measured on the day of discharge was significantly higher in the four patients. Additionally, there was a significant difference in ferritin levels between the control group and the survivors after two weeks and a month of follow-up. Also, ferritin levels were associated with the persistence of general fatigue among the surviving patients after their discharge from the hospital. All of this indicates the importance of ferritin in predicting the risk of infection and death.

Regarding sex and smoking, the findings of our study also revealed that male patients had a greater incidence of COVID-19 (62.2%) than female patients (37.8%), which is consistent with earlier studies [27–29]. This can be understood by factors such as, diabetes, male sex hormones, and immunodeficiency, which are higher in men than women, also the role of protective health behaviors (hand washing, wearing a face mask, and physical distance), which are more prevalent in women than men [30-32]. Additionally, despite the fact that smokers are more prone to develop respiratory ailments, in our study there were more smokers in the group of survivors. According to several research, smokers express more angiotensin protein in their airways than non-smokers do. However, it has been proposed that other factors associated with smokers' habits and behaviors are to blame for the overexpression of the ACE2 protein in smokers. according to one theory, nicotine inhibits SARS-COV-2 entry into cells by competing with the virus' spike proteins for binding to the acetylcholine receptors, which serve as the virus' binding site [33]. Another possible explanation is that nicotine may reduce systemic inflammation by blocking the release of proinflammatory cytokines. This is because of activating the pathway of anti-inflammatory cholinergic which can counteract intense inflammatory responses is mediated by acetylcholine receptors [34]. In addition, some researchers have speculated that the tobacco mosaic virus commonly circulate in the airways of smokers which stimulates innate and adaptive immunity that may have a protective effect against COVID-19 infection [35].

When CK-MB and AST levels were compared between patient group and the control group, a significant difference was discovered. Also, 42.2% of the patient group had increased CK-MB and AST levels that were higher than the maximum values for the control group. Which indicates the role of the virus in the possibility of causing damage to heart cells in patients with Covid-19. Compared to other studies, a Korean study found that 15.6% of the patients had high CK-MB levels above normal value [36]. This may be due to the high prevalence of cardiovascular diseases in Syria, as it was the first cause of death in our country for several consecutive years [37]. Heart injury in COVID-19 patients may occur due to several reasons such as that ACE2 is highly expressed in the cells of adult human hearts, indicating the possibility of direct cardiac infection with SARS-CoV-2. In addition, the patients with heart failure disease have an increased expression of ACE2, and might have higher possibility of severe heart injury after infection [38]. It was shown that the initial entry of SARS-CoV-2 through ACE2 leads to reduced expression of ACE2 which leads to reduced conversion of angiotensin II (Ang-II) to angiotensin 1-7 (Ang-1-7). Ang-1–7 play role in reversing the effect of the proinflammatory, vasoconstrictor, proproliferative, profibrotic actions and pro-oxidant exerted by Ang-II via angiotensin II receptor type 1 (AT1) receptors [39,40]. In addition, microangiopathy, cytokine storm, endothelial dysfunction, Ang-II upregulation and oxidative stress, and the thrombotic disorder that may occur in severe cases of COVID-19 may explain the heart damage that occurred in these patients [41]. This result is very important, as heart diseases were at the forefront of the causes of death in Syria, and thus the possibility of heart disease due to COVID-19. is expected and dangerous at the same time. It is important to seek early diagnosis of cardiac damage, which is likely to occur at a high rate in Syrian patients with COVID-19.

In addition, when assaying CK-MB, AST of the patients after discharge, no significant difference was observed between these parameters and the parameters of the control group. However, it was found that there was a significant increase in ferritin levels up to a month after discharge from the hospital. This was also associated with persistence of several clinical symptoms for patients, mainly general fatigue. Hyper-ferritinemic syndrome, which was found in our study, is a complex occurrence may be induced to cellular apoptosis. Iron metabolic disorder in COVID-19 infection may not only be exclusive to digestion of hemoproteins and resultant iron cell leakage, but also to direct and indirect metabolic affected by infection [42]. Some studies also suggest that ferritin levels increased in response to inflammation concomitantly with the increase of cytokines as IL-6 and IL-1ß, primarily because these molecules activate the synthesis of hepcidin [43]. Interestingly, SARS-CoV2 has hepcidin-mimetic action which increases ferritin levels [44]. However, as SARS-CoV-2 relies on the cell's metabolic to replicate, the virus has developed strategies to maximally utilize the resources of host. Our study shows the relation of patient clinical symptoms with blood markers of inflammation disarrangement after the acute virus infection and this suggests ongoing change in the host metabolism, which causes these symptoms to continue after the acute virus infection.

5. Conclusions

This is the first prospective multicenter study in Syria that showed the importance of checking both ferritin and cardiac enzymes levels before deciding whether or not to discharge patients from hospitals, as we came to the conclusion that ferritin is a crucial marker for predicting death, while CRP played the biggest role in disease diagnosis. Additionally, ferritin was linked to the persistence of some patients' clinical complaints, including general fatigue, which was the most permanent clinical symptom among patients after leaving hospitals.

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