

Serum levels of interleukin – 6, ferritin, C-reactive protein, lactate dehydrogenase, D-dimer and count of lymphocytes and neutrophils in COVID-19 patients. Its correlation to the disease severity.

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ABSTRACT

Since its first emergence in Wuhan city, China, SARS CoV2, which is responsible for the pandemic COVID-19, has become a significant health problem all over the world affecting over 2.1 million people globally. The current study aimed to investigate serum levels of IL-6, ferritin, CRP, LDH, D-dimer as well as lymphocytes and neutrophils count in COVID-19 patients, and to clarify the correlation of these parameters with disease severity and progression. For these purposes, (100) patients with COVID-19 (confirmed by PCR) and (20) apparently healthy people (with matched age and sex) were included in the current study and considered as a control group. All study population (patients and control) were subjected to the evaluation of serum levels of IL-6, ferritin, CRP, LDH, D-dimer, as well as lymphocytes and neutrophils, count. COVID-19 patients showed a significant elevation in the levels of all parameters included in this study when compared with healthy subjects. We also found that all of IL-6, ferritin, CRP, LDH, D-dimer are significantly associated with the severity of the COVID-19 symptoms. lymphopenia and increased neutrophils were also effectively correlated with disease progression. In line with these results, we concluded a proportional correlation between the aforementioned parameters and COVID-19 suggesting the uses of these tests to the diagnosis of critical cases.

Key words: COVID-19. IL-6, ferritin, CRP, D-dimer, lymphocyte, neutrophils.

INTRODUCTION

Since its first emergence in Wuhan city, China at the end of 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive sense single-stranded enveloped virus which is responsible for the pandemic COVID-19 has become a significant health problem all over the world with over 2.1 million cases and 120,000 deaths so far [1]. In Iraq, the first case of COVID-19 was recorded in March 2020 in Al Najaf city, since which the virus spread dramatically around the country causing approximately 51300 infections and more than 7000 deaths according to the daily reports declared by the Iraqi ministry of health.

The clinical spectrum of SARS-CoV-2 infection appears to be wide, encompassing asymptomatic infection, mild upper respiratory tract illness, and severe viral pneumonia with respiratory failure and even death, with many patients being hospitalized with pneumonia [2].

Urgent identification of clinical and laboratory predictors of progression to serious and lethal forms is urgently needed in the battle against COVID-19 disease. These predictors will allow clinical management, direct inductive research approach to target patients at increased risk of developing a serious disease and optimize the allocation in the ongoing pandemic of limited human and technological resources. Besides, the identification of laboratory criteria capable of distinguishing between severe and non-serious cases, or those at high or low mortality risk, would enable the enhancement of clinical understanding of the situation [3].

There is overwhelming evidence that there are features of hyper inflammation in critically ill patients, consisting of elevated serum C-reactive protein (CRP), procalcitonin (PCT), D-Dimer, and hyperferritinemia [4]. Furthermore, lymphopenia is strongly correlated with the seriousness of the disease. Patients who died from COVID-19 have substantially lower lymphocyte counts than survivors. Other blood cells, such as neutrophils, eosinophils, platelets, as well as CD8+ cell counts, have been partially considered as determinants in discriminating mild from severe COVID-19, the significance of which is still vague [5]. Cytokine storms may also be related to disease severity. Increased serum expression of interleukin (IL)-2R and IL-6 appears to be predicting the severity and prognosis of COVID-19 patients. Pathological analysis of a biopsy sample from a patient who died from COVID-19 showed interstitial mononuclear inflammatory infiltration in both lungs. Also, the peripheral cytometric examination of blood flow revealed that overactivation of T cells compensated, in part, for the extreme immune injury in this patient. These results indicate a potentially important role in the pathophysiology of the cytokine storm COVID-19 [6].

In line with the aforementioned, this study aimed to estimate serum levels of IL-6, D dimer, ferritin, C-reactive protein as well as total blood lymphocyte counts in patients with COVID-19 disease, in addition, to explore the correlation between the levels of the above parameters and severity of the disease. To our knowledge, this is the first study in Iraq that considers such goals in patients infected with SARS-CoV-2.

MATERIALS AND METHODS

Study population

The current study involved (100) patients suffering from COVID-19, aging between (18 - 45) years old and of both sexes. Also, (50) apparently healthy persons of the same ages and sexes were included in this study as a control group. COVID-19 patients were grouped into two groups (simple to moderate and severe) according to their symptom's severity (fever, cough, respiratory distress, loss of sense of taste and smell, and needing for ventilation). All of the patients and healthy persons were subjected to the estimation of serum IL-6, D-Dimer, lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, as well as the total count of lymphocyte and neutrophils.

Study Sampling

5 ml of venous blood is collected from the study groups and divided into two tubes (plane tubes and EDTA tubes). The first part of the blood samples (EDTA tubes) is applied to the

hematology autoanalyzer (Pentra 80. manufactured by the ABX-Horiba group, Minami-Ku Kyoto Japan). The second part of the blood samples was centrifuged to obtain serum which is used for the determination of serum concentration of IL-6, D-Dimer, lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin.

Estimation of serum IL-6:

Electrochemiluminescence immunoassay (ECLIA) technique and (Cobas) instrument are used for the detection of serum IL-6 in patients and the control group by using a kit performed by Roche company (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim. 2020).

Determination of serum D-Dimer and C-Reactive protein

Serum concentrations of D-Dimer and C-Reactive protein were evaluated by using a specific automated protein analyzer (PA120) provided by (Shenzhen Genius Electronics Co., Ltd. China 2019). Serum samples for each of the patients and healthy persons were applied to the instrument then the concentrations of D-Dimer and C-Reactive protein are calculated automatically.

Total leukocytes and neutrophils counting:

Anticoagulated blood samples from both patients and the control group are applied to a hematology autoanalyzer (Pentra 80. manufactured by ABX-Horiba group, Minami-Ku Kyoto Japan) to estimate total counts of WBCs and neutrophils. Samples are processed by the instrument then total WBCs and neutrophils are calculated automatically.

Statistics:

The results of the current study are analyzed using SPSS software package version 20. to find the variation in the results of the study. Statistical significance was defined as a (P -value < 0.05). Statistical tests such as (independent T-test, percent) were used for data analysis.

RESULTS

The results of the current study, which included (100) patients suffering from SARS CoV2 infection, found, as illustrated by figure (1), that the percent of COVID-19 infection was higher in women (60%) than men (40%).

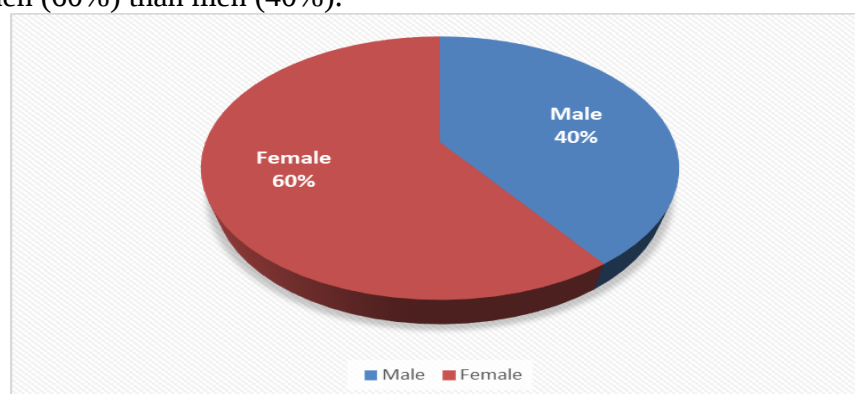


Figure 1. Percent of COVID-19 infection according to sex

Our results also found, as shown in table (1), that the mean of the serum level of IL-6 was elevated significantly (P -value ≤ 0.000) in COVID-19 patients (26.72 ± 15.05 pg/ml) compared to the control group (5.38 ± 0.89 pg/ml). Likewise, the mean of the serum levels of CRP, ferritin, LDH, and D-Dimer was also increased significantly among COVID-19 patients compared to the control group.

Table 1. The differences in the serum levels of IL-6, ferritin, LDH, CRP and D-Dimer in COVID-19 patients and control group.

Parameters	Patients Mean $\pm$ Std. deviation	Control Mean $\pm$ Std. deviation	P value
IL-6 (pg/ml)	26.72 $\pm$ 15.05	5.38 $\pm$ 0.89	≤ 0.000
Ferritin (μ g/ml)	447.65 $\pm$ 210.62	70.71 $\pm$ 38.32	≤ 0.000
CRP (mg/ml)	110.24 $\pm$ 71.26	2.73 $\pm$ 0.92	≤ 0.000
LDH (IU/L)	432.78 $\pm$ 103.51	137.85 $\pm$ 19.88	≤ 0.000
D-Dimer (μ g/ml)	1.83 $\pm$ 0.99	0.21 $\pm$ 0.06	0.041

The hematologic findings of this study revealed that total lymphocyte count was decreased significantly (P value=0.018) in patients infected with SARS CoV2 (mean = 13.66 $\pm$ 4.29), in contrast, neutrophils count was increased significantly (P-value $\leq$ 0.000) in patients (90.43 $\pm$ 12.40) compared with healthy subjects (51.63 $\pm$ 5.66), see table (2).

Table 2. The total count of lymphocytes and neutrophils in COVID-19 patients and control group.

Parameters	Patients Mean $\pm$ Std. deviation	Control Mean $\pm$ Std. deviation	P value
Lymphocyte (cell / ml)	13.66 * 10 ³ $\pm$ 4.29 * 10 ³	26.40* 10 ³ $\pm$ 4.79* 10 ³	0.018
Neutrophils (cell / ml)	90.43 * 10 ³ $\pm$ 12.40* 10 ³	51.63* 10 ³ $\pm$ 5.66* 10 ³	≤ 0.000

Considering to the symptom's severity, table (3) demonstrates that all of IL-6, ferritin, LDH, and CRP, except D-Dimer, are associated with severity of SARS CoV2 infection, the levels were significantly higher up to (28.63 $\pm$ 14.67 pg/ml, 471.60 $\pm$ 202.92 μ g/ml, 119.98 $\pm$ 68.54 mg/ml, and 443.55 $\pm$ 99.38 IU/L) for IL-6, ferritin, RRP, and LDH respectively in patients with severe infection. On the other hand, patients with simple to moderate infection, showed approximately normal levels of IL-6, ferritin, CRP, and LDH (9.56 $\pm$ 0.66 pg/ml, 232.14 $\pm$ 161.29 μ g/ml, 25.64 $\pm$ 22.53 mg/ml, and 335.80 $\pm$ 97.63 IU/L) respectively. However, D-Dimer level was found to decreased non significantly (P value= 060) in a patient with severe infection (1.68 $\pm$ 0.30) compared to a patient with mild to moderate symptoms, table (3).

Table 3. Differences in the levels of IL-6, ferritin, LDH, CRP, and D-Dimer in COVID-19 patients according to the severity of infection

Parameters	Simple to Moderate Mean $\pm$ Std. deviation	Sever Mean $\pm$ Std. deviation	P value
IL-6 (pg/ml)	9.56 $\pm$ 0.66	28.63 $\pm$ 14.67	0.006
Ferritin (μ g/mL)	232.14 $\pm$ 161.29	471.60 $\pm$ 202.92	0.014

CRP (mg/ml)	25.64±22.53	119.98±68.54	0.004
LDH (IU/L)	335.80±97.63	443.55±99.38	0.026
D-Dimer (µg/mL)	0.48±0.61	1.68±0.30	0.60 ^{NS}

In the same context, table (4) reveals that lymphocytes are decreased significantly (P value= 0.012) in patients with severe COVID-19 disease contrary to neutrophils which was increased significantly (P-value= 0.013) in the same patients.

Table 4. Differences in lymphocytes and neutrophils count in COVID-19 patients according to the severity of infection

Parameters	Moderate Mean ± Std. deviation	Sever Mean ± Std. deviation	P value
Lymphocyte (cell / ml)	18.14*10 ³ ± 3.29*10 ³	13.16*10 ³ ± 4.12*10 ³	0.012
Neutrophils (cell / ml)	77.04*10 ³ ± 12.05*10 ³	91.26 *10 ³ ± 12.28*10 ³	0.013

DISCUSSION

In the present study, which is conducted in Baghdad city, 60% of the SARS CoV2 infected patients were women and 40% were men. In agreement with our results, Gebhard *et al.*, (2020) found that female's percent of infection were higher than males and reached (52%) in Switzerland and Belgium, (54%) in Portugal, (53%) in France, and (60%) in South Korea (7).

Females and males have a variable response to viral infection just like SARS CoV, MERS CoV, SARS CoV2, and other viruses. These differences are the leading of disease severity and incidence between the two genders. Multiple factors contribute to the disparity in sex-specific disease outcomes following virus infections. Sex-specific steroids and the activity of X-linked genes, both of which modulate the innate and adaptive immune response to virus infection, influence the immune response. Furthermore, the differences in the expression of angiotensin-converting enzyme (ACE) 2 receptor and the cellular serine protease TMPRSS2, which are necessary for the binding and priming of SARS CoV2, may have an important role (7,8). We also believe that the increased stress and domestic work during the pandemic especially in Iraq may also affect the capabilities of the female immune system against COVID-19.

There is a significant increase in the serum levels of IL-6, ferritin, CRP, LDH, and D-Dimer in COVID-19 patients (Table 1). Also, the levels of these parameters are effectively associated with the diseases severity in the patients as shown in (Table 3). Zhu *et al.*, 2020 (9), reported that IL-6 is elevated in COVID-19 patients and reached to (24.11 pg/ml) and its level was significantly correlated with the severity of the symptoms in COVID-19 patients. Similarly, Huang *et al.*, 2020 (10) found that IL-6 is increased in patients with SARS CoV2 infection and its high level was closely associated with the level of virus RNA in the blood and disease progression. It is stated that during viral infection, IL-6 together with transforming growth factor-beta induces the differentiation of naïve CD4 into Th17 cells, which are important for the defense against viruses and other pathogens at mucosal sites. Also, there is synergic interaction between IL-6 and IL-7 and IL-15 to induce the differentiation and cytolytic ability of CD8 T cells which is important in the response against viral infections (11).

The ferritin level in COVID-19 patients, as seen in the table (1), was significantly increased and reached to ($447.65 \pm 210.62 \mu\text{g/mL}$). Likewise, the serum level of CRP was also elevated significantly in patients when compared with the healthy group. The elevation of ferritin and CRP was correlated with the disease progression (Table 3). Several publications have shown that elevated ferritin levels have been associated with worse outcomes, along with several other pro-inflammatory markers, involving CRP and IL-6, and may even help predict these outcomes (12-14). Elevated ferritin levels due to secondary haemophagocytic lymphohistiocytosis and cytokine storm syndrome have been reported in severe COVID-19 patients. It has been proposed that COVID-19 could be part of the broader spectrum of hyperinflammatory syndromes such as the secondary haemophagocytic lymphohistiocytosis (15). Hyperferritinemia is a cardinal characteristic of these syndromes. COVID-19 severity and worse prognosis mean that mortality could be due to virally induced hyper inflammation which is substantially associated with high ferritin level. However, the circulating levels of ferritin can not only represent a strong phase response but play an important role in inflammation (16). In COVID-19 patients, CRP levels were increased and it was shown that survivors had a median CRP of approximately (40 mg / L), while non-survivors had a median CRP of (125 mg / L), suggesting a good association with the severity and prognosis of the disease (17).

In addition to IL-6, ferritin, and CRP, our results also found a significant elevation in the level of LDH as well as D-Dimer in COVID-19 patients. However, the increases in both D-Dimer and LDH were effectively correlated with disease severity as shown in (Table 3). D-Dimer is a commonly used fibrin degradation test used to diagnose thrombotic diseases at an early level (18). Prior studies have shown that D-Dimer level is higher in extreme cases of community-acquired pneumonia and chronic obstructive pulmonary disease and can be used as a significant biomarker and that D-Dimer $> 1 \mu\text{g / mL}$ is one of the mortality risks factors in adults with COVID-19 who receive the medication for their pneumonia in a group acquired environment (19, 20). It was also stated that D-Dimer at admission more than $2.0 \mu\text{g / mL}$ (fourfold increase) could effectively be correlated with disease severity and mortality in Covid-19 patients, as shown in table (3) in this review. These results indicated that D-Dimer could be an early and helpful marker to improve the management of Covid-19 patients (18,21,22).

LDH is a glycolytic cytoplasmic enzyme present in virtually every tissue. Generally, its elevation suggests tissue injury. In patients infected with MERS-CoV, increased LDH was a typical finding in (23,24). Independent mortality factors have been identified for patients with the extreme acute respiratory syndrome (25) and H1N1 infection (26). Possible subclinical tissue damage was indicated by our observation of increased LDH in the early stage of extreme COVID-19 cases. Although the virus binds to the human angiotensin-converting enzyme 2 (ACE2) receptor in the lungs (27,28), which explains why the lungs are the first affected organs, different cytokine abnormalities and multiple organ dysfunction can be observed in serious patients as the disease progresses, suggesting systemic organ damage caused by excessive immune system activation (29, 30).

Results of table (2) showed that COVID-19 patients have significantly decreased lymphocyte count (P value= 0.018) and significantly increased neutrophils count (P-value $\leq$ 0.000). the decreased and elevated lymphocyte and neutrophils count was significantly correlated to the disease severity as seen in the table (4).

Hematologic variations were established as supporting evidence of infection with COVID-19 and as potential signs of serious illness. Numerous clinical guidelines define that potential SARS-CoV-2 infection, especially in severe cases, exhibits irregularities in

hemocytometry. The U.S. Centers for Disease Control and Prevention have issued guidelines emphasizing that the most common laboratory abnormalities recorded in hospitalized COVID-19 patients with pneumonia were leukopenia (92-5%), leukocytosis (24-30%), and lymphopenia (63%) (31).

Lymphopenia was also a clear finding and experiments using flow cytometry showed that these improvements were correlated with lower counts of CD4⁺ and CD8⁺ T lymphocytes (32, 33). In the same context, Song et al 2020 (34) have revealed that the most consistent abnormal hemocytometric findings in COVID-19 patients are lymphopenia and increased neutrophils, and these changes can also increase throughout the disease, especially in those with serious illness. In pregnant women with COVID-19, lymphopenia was also reported, however, this finding is even less significant in infected children (35-37).

CONCLUSION

Contrary to previous studies, our current study found that male sex may be at low risk to be infected with SARS CoV2. Infection with COVID-19 has led to significant elevation in the levels of IL-6, ferritin, CRP, LDH, D-dimer as well as lymphopenia and increased neutrophils count. These differences were effectively associated with disease severity and progression, suggesting the use of such clinical markers to recognize severe illnesses.

ETHICAL CLEARANCE

Permission to conduct this study was issued by the Health institutional and the collection of Blood samples of individuals was carried out by under public health technician supervision.

CONFLINT OF INTEREST

All authors declare that there is no any conflict of interest.

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