ORIGINAL PAPER

The Relationship of Some Biochemical Markers with Inflammatory Prognosis in COVID-19 Patients

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Abstract

Background and objectives: For COVID-19 patients who need effective management, it is necessary to identify the laboratory biomarkers of progression approaching morbidity and mortality. This study aims to demonstrate numerous biochemical tests that seriously afflicted individuals. Such as ferritin, Interleukin-6 (IL6), D-dimer, Uric acid (UA), Lactate dehydrogenase (LDH), and C-reactive protein (CRP) have been proposed as disease biomarkers. **Materials and methods:** Blood samples were collected from 30 healthy individuals. In addition, other 60 patients confirmed by RT-PCR as COVID-19 positive after 3 to 5 days followed up with them every five days for fifteen days from the beginning of symptoms for the above parameters. All patients attended Isolated Hospital in Tikrit City / Iraq from February to June 2021. **Results:** Ferritin, D-dimer, IL6, CRP, LDH, and UA gradually increased to the maximum mean (1138±782.7, 1155±400.42, 23.28±4.73, 104.86±53.69, 424.91±95.4 and 5.94±1.765.94±1.76) respectively after (8-10) days and return to normal range after 15 days. **Conclusion:** There is a significant association of these parameters with inflammatory prognosis in COVID-19 patients.

Keywords: Ferritin, C-reactive protein, D-dimer, Interleukin-6, Lactate dehydrogenase.

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INTRODUCTION

The advent of coronavirus infection in 2020 has been a watershed moment (COVID-19). It is a pandemic that has infected numerous nations, paralyzing the people's lives were compelled them to restrict themselves at home. Direct SARS-CoV-2 attacks and medical problems mediated by overactive inflammatory responses might cause the illness progression[1].

C-reactive protein (CRP) is a widely observed inflammatory biomarker. It is elevated in many COVID-19 subjects and linked to disease activity. In some comprehensive researches[2,3], the CRP was indicated as the basic, early measure for prognosis during intensive care unit (ICU) hospitalizations for septic cases. It had been demonstrated to be related to chronic inflammatory disorders, like cardiovascular illnesses and diabetes mellitus type II, as well as being an indicator of acute inflammation[4,5].

CRP is a hepatocytes-generated protein, and it can be triggered by proinflammatory cytokines such as IL-6[6]. In COVID-19 subjects, high serum IL-6 and ferritin levels were indicated as prognostic markers[7]. Given that these indicators were examined during the initial stages of the crisis and that the majority of the reports were retrospective in nature, it's possible that the lethality of the COVID-19 virus has changed as a result of human-to-human transmission[8,9].

Ferritin seems to be an intracellular formed protein that stores iron and is essential in inflammatory disorders like neurodegeneration, infection, and cancer. According to certain studies, ferritin is a remarkable predictor of COVID-19 aggressiveness in individuals. Furthermore, there is an unknown link between patient mortality and ferritin[10-12]. However, the values of such cytokines were significantly greater in severe conditions compared to moderate ones[13], suggesting that early severity prediction requires IL-6 monitoring[14,15].

D-dimer is just a fibrin breakdown product that is commonly employed as a thrombotic disease biomarker. When the incidence of society pneumonia increases, so does the concentration of D-dimer[16]. D-dimer was already found as a possible indication for COVID-19 patients' prognosis since the start of the pandemic. Numerous reports show that the admitting day D-dimer can help predict illness severity[17,18]. COVID-19 management may benefit from accurate and easily obtainable prognostic markers[19,20].

Lactate dehydrogenase (LDH) is an intracellular enzyme that catalyzes the conversion of lactate and pyruvate. In addition, the conversion of NAD+ and NADH in basically most organs system. Two main subunits make up the enzyme (A and B). LDH levels rise during the initial stages of myocardial infarctions and hemolysis. The liver, erythrocytes, striated muscles, heart, brain, kidneys, and lungs are the organs where it is most active[21]. COVID-19 is a virus that can harm vital organs such as kidneys, liver, and heart[22] more than other organs[23]. High LDH values of seriously sick individuals have been linked to increased activity and the amount of lung damage, according to COVID-19[24]. According to previous research, LDH is unlinked to a bad prognosis[25,26].

The most prevalent antioxidant compound in the bloodstream is serum uric acid (SUA). Individuals with high SUA quantities have an evolutionary benefit that may improve antioxidant defenses and lengthen life. In healthy subjects, the uric acid (UA) infusion raises SUA concentrations and boosts the serum antioxidant activities[27]. The SUA may protect the brain[28], as well as glaucoma[29], from oxidative damage effects[30].

MATERIALS AND METHODS

The medical ethics committee of Salah Aldeen Health Directorate approved this study with a code number (IQ.TIK.1975.21). All participants signed an ethical acceptance form based on the World Medical Association Declaration of Helsinki, amended in 2000, Edinburgh.

A case-control study contains 60 individuals. The RT-PCR was used for the diagnosis of as positive COVID-19 cases after (3-5) days from the appearance of the signs and symptoms like fever, cough, fatigue, and headache. Then follow up with them every five days for 15 days from the beginning of the symptoms for all parameters. All individuals were confirmed as positive for COVID-19 infection with the pharyngeal swab done by RT-PCR. Blood samples were collected from all 60 patients attended to the Isolated Hospital in Tikrit City / Iraq from February to June 2021. Also, 30 samples of healthy individuals to estimate the levels of Ferritin, D-dimer, IL6, and CRP by using ELISA kits (Sunlong Company)[31] and measured LDH and Uric acid by using enzymatic methods (Biolabo kits). All individuals ranged from (30-60) years. They were not smokers, not diabetics, with negatively personal and family history of diabetes, hypertensive, thyroid, renal diseases, or any other diseases.

The statistical analysis was carried out using SPSS, version 26 statistical programs, and a comparison between several groups was undertaken, with the t-test to analyze the results. The statistical significance threshold was determined to be (P<0.05).

RESULTS

According to Table (1), the Mean±SD of all parameters every five days from signs and symptoms of Covid-19 infection appearance, the results were increased after (8-10) days with highly significant differences (P≤0.01) rather than after (3-5) days, after (13-15) days and control; except Uric acid which maximally increased after (13-15) days.

Table 1. The Mean±SD of all parameters every 5 days from Covid-19 Infection

Tests	After (3-5) days	After (8-10) days	After (13-15) days	Control
Ferritin ng/ml	514.7±245.8	1138±782.7	480.6±193.4	183±84.8
D-dimer ng/ml	868±266.7	1155±400.42	512±183.9	180±60.82
CRP mg/L	45.75±21.8	104.86±53.69	25.7±10.5	4.3±0.98
IL6 pg/ml	18.2±6.2	23.28±4.73	16.9±6.8	15.8±10.32
LDH IU/L	328.5±76.7	424.91±95.4	364.6±80.6	240.2±35.36
UA mg/dl	4.6±1.6	5.94±1.76	6.5±2.2	4.5±0.97

There was a highly significant difference (P≤0.01) between all parameters for all stages of Covid-19 infection when compared with control except in IL-6 (after (3-5) days and after (13-15) days) while uric acid after (3-5) days with no significant difference (P > 0.05) showed in Table 2.

Table 2. The P value of all parameters every 5 days from Covid-19 Infection and compered with control.

Tests	After (3-5)days	After (8-10)days	After (13-15)days
Ferritin ng/ml	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01
D-dimer ng/ml	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01
CRP mg/L	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01
IL6 pg/ml	P = 0.172	P ≤ 0.01	P = 0.546
LDH IU/L	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01
UA mg/dl	P = 0.754	P ≤ 0.01	P ≤ 0.01

According to Figure 1, the levels of ferritin were increased after (3-5) days from signs and symptoms of Covid-19 infection appearance that gradually increased after (8-10) days. Furthermore, it decreased rather than the beginning after (13-15) days but without returning to the control level.

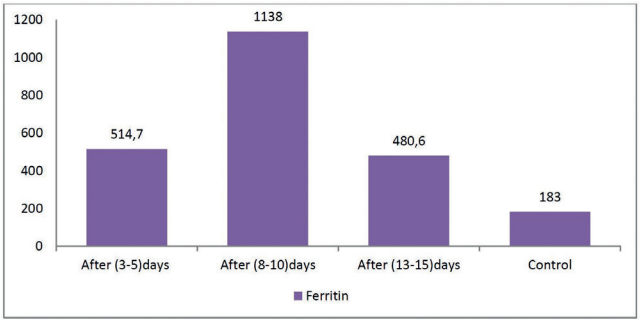

Figure 1. The levels of Ferritin every 5 days from Covid-19 Infection.

According to Figure 2, the levels of D-dimer were increased after (3-5) days from Covid-19 infection and gradually increased after (8-10) days. On the other hand, it decreased rather than at the beginning after (13-15) days without returning to the control level.

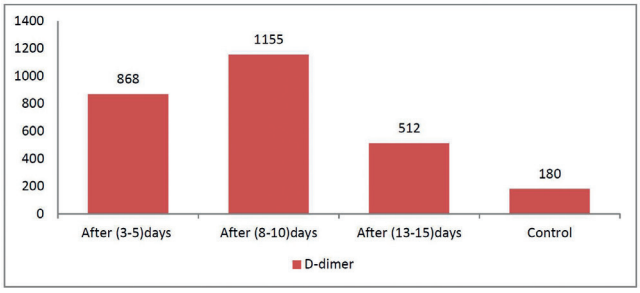

Figure 2. The levels of D-dimer every 5 days from Covid-19 Infection.

According to Figure 3, the levels of CRP were increased after (3-5) days from signs and symptoms of Covid-19 infection appearance and gradually increased after (8-10) days. On the other side, it decreased rather than the beginning after (13-15) days without returning to the control level.

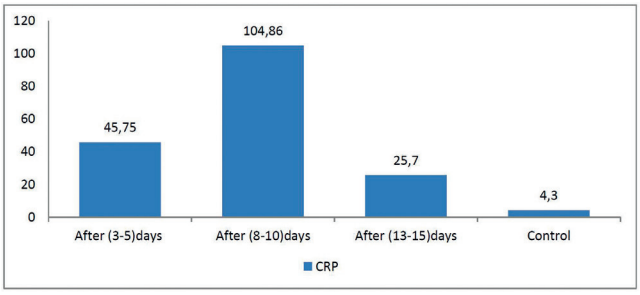

Figure 3. The levels of CRP every 5 days from Covid-19 Infection

According to Figure 4, the levels of IL-6 were increased after (3-5) days from signs and symptoms of Covid-19 infection appearance and gradually increased after (8-10) days. Furthermore, it decreased rather than at the beginning after (13-15) days but without returning to the control level.

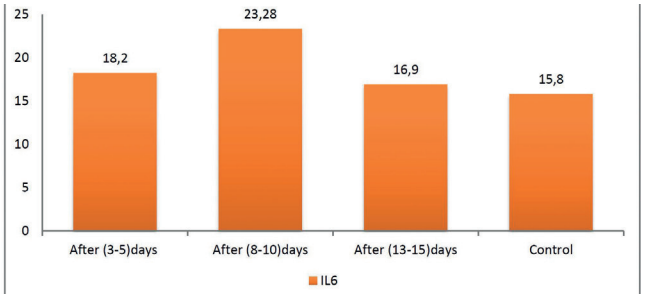

Figure 4. The levels of IL-6 every 5 days from Covid-19 Infection.

According to Figure 5, the levels of LDH were increased after (3-5) days from signs and symptoms of Covid-19 infection appearance and gradually increased after (8-10) days. On the other hand, it increased rather than at the beginning after (13-15) days without returning to the control level.

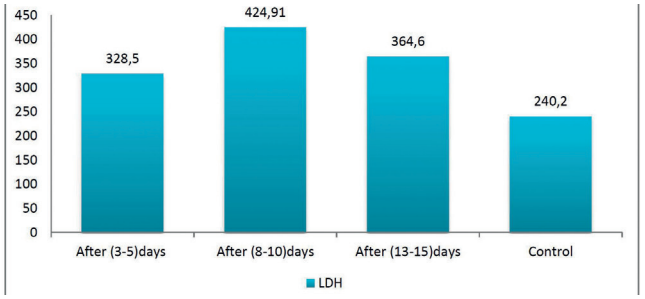

Figure 5. The levels of LDH every 5 days from Covid-19 Infection.

According to Figure 6, the levels of uric acid were slightly increased after (3-5) days from Covid-19 infection and gradually increased until reach to the maximum level after (13-15) days but without returning to the control level.

Figure 6. The levels of Uric acid every 5 days from Covid-19 Infection

DISCUSSION

The levels of ferritin, D-dimer, CRP, IL6, and LDH gradually increased after (3-5) days from starting the signs and symptoms of COVID-19 infection and reached the maximum levels after (8-10) days. In spite of that, it decreased after (13-15) days before reaching to normal range. While the levels of uric acid increased after (13-15) days before reaching to normal range. The values of all clinical testing results of patients were evaluated in this study, notably in critical and severe patients admitted intensive care unit (ICU), and a retrospective analysis was undertaken to assess the prognostic utility of them in death rates in hospitalized COVID-19 patients.

This might support the idea that assumes hyperferritinemia is linked to immune activation in SARS-CoV-2 virus infection, and that ferritin would be used to estimate illness severity and the size of the cytokines storm. Furthermore, the cause of the elevated plasma ferritin amount, as well as the protein's possible function in inflammation shortly after the onset of COVID-19 illness, remains unknown[32]. Active ferritin formation can occur during the progression of inflammatory disorders. The release of serum ferritin might be attributed to macrophages, which generate cytokines and make up the bulk of immune cells within the lung tissues. Ferritin production can also be triggered by inflammatory stimuli such as cytokines like IL-6[33].

Interestingly, increased IL-6 levels are linked to illness severity in COVID-19 individuals. According to previous research, the immunological description of critically sick Covid-19 patients is directly linked to

the over-activity of the humoral immune path. Also, together with IL-6 as a crucial mediator for shock, respiratory failure, and multiorgan impairment[34].

In COVID-19 patients, endothelial dysfunction is mainly limited to the lungs as they are the first and most commonly affected organs in these patients. D-Dimer is an indirect marker of thrombus formation that increases in COVID-19 patients suggesting the development of thrombus, particularly in ARDS patients, indicating poor prognosis. However, as an indirect marker, a significant increase in D-Dimer suggested a benefit from heparin infusion in a large group of ARDS patients[35,36].

CRP levels may diagnose severe pneumonia caused by COVID-19. On the other side, there are many blood indicators that tend to be associated with the severity of SARS-CoV-2 infection and death. The level of CRP was dramatically elevated in highly infected SARS-CoV-2 patients[37].

In the damaged cells, LDH is secreted from inside. The level and activity of LDH throughout the blood increase[38]. Many shreds of evidence assume that serum LDH levels can be used as a non-specific indication of cell death in different illnesses[39]. Excessive LDH implies tissue hypoperfusion, which might reflect the disease severity and alter the prognosis[40]. Early results from COVID-19 individuals revealed substantial variations in LDH values between patients and those who did not have the severe condition[41].

CONCLUSION

This study showed a significant association between ferritin, D-dimer, IL6, CRP, LDH, and UA with the inflammatory prognosis process in COVID-19 patients.

Compliance with ethics requirements: The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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