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Evaluation of the Activity of Metalloendopeptidase enzyme, Interleukin-6 level and Some Physiological Variables in the sera of patients infected with SARS COVID-2

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Abstract

The current study included a collection of 60 serum samples, 40 samples for patients infected with Coronavirus collected from Health centers and outpatient medical clinics in Kirkuk, and 20 samples for healthy people. This paper involves the evaluation of D-dimer, C-reactive protein, Metalloendopeptidase, Interleukin-6 Concentration, white blood cells, and lymphocytes.

The present study's results demonstrated a considerable increase in the levels of plasma D- dimer, C-reactive protein, and IL-6 in the group serum infected with COVID-19 compared to healthy individuals. They also identified a considerable increase in the activity of the enzyme metalloendopeptidase in the blood serum of COVID-19-infected people compared to healthy people. The data also demonstrated WBCs in the blood of infected individuals significantly increased. Also, the result was found that C-reactive protein, di-dimer. And IL-6 is highly sensitive to detecting infection with the COVID-19 virus.

Keywords: Coronavirus, Metalloendopeptidase, Interleukin-6, D-dimer, C-Reactive Protein

1. Introduction

Since coronavirus COVID-19 is a closed, single-chain RNA virus, its RNA polymerase enzymes are not attached to the ribonucleic acid but are instead encoded in genes with bacilli-like extensions., these viruses are named Coronavirus according to the Latin corona, which means crown [1, 2]. Coronaviruses are classified under the Orthocorona Viridian subfamily, due to the fact that there are four main groups within the Orthocorona Viridi subfamily, each of which contains numerous further subgroups, Humans, bats, pigs, and cats all carry viruses that fall into the categories of beta, alpha, delta, and gamma coronaviruses. As well as dogs, mice, and birds [3, 4].

Symptoms typically start between two and fourteen days after exposure to the virus, which spreads fast from person to person by respiratory droplets formed during coughing and sneezing. Fever, exhaustion, and a dry cough are the Covid-19 disease's most typical symptoms. Aches and pains, a stuffy nose, a cold, a sore throat, or diarrhea are some of the symptoms that some people may have; these conditions are typically mild and develop gradually. Some people contract an infection even though they have no symptoms or feel ill [5].

As a result of inflammation brought on by exposure to COVID-19, a rise in the level of C-reactive protein (CRP) has been linked to various biochemical changes during infection [6].

In addition to high levels of D-dimer, which is a potential indicator for diagnosing complications of the disease, many studies have [7] own promising results for predicting disease severity [8, 9] with COVID-19. Which can predict further clinical course and associated systemic complications, thus helping the clinician to assess the clinical condition and disease progression and plan treatment for the patient [10].

The Metalloendopeptidase enzyme is part of the family of beta-adidases EC 3.4.24.11. It belongs to the German hydrolytic enzymes, and the general name of the enzyme is metalloendopeptidase. The Metallo syllable indicates that the enzyme is a mineral enzyme, as it contains zinc as a co-factor [11] while the endopeptidase refers to the work of the enzyme to break the peptide bonds of peptides and proteins inside the peptide, while the syllable Endopeptidase refers to breaking the peptide bonds of peptides and proteins inside the peptide.

The enzyme has many names, including Kidney brush border metalloendopeptidase, Enkephalinase, and Neutral metalloendope [12]. Therefore, the current study sought to assess the effect of interleukin-6 and the activity of the metalloendopeptidase enzyme in the blood serum of SARS-COVID-2 patients.

2. Material and methods

This study involved the collection of 60 serum samples, including 20 samples from healthy individuals and 40 samples from COVID-19-infected patients from Kirkuk's health centers and outpatient clinics. The age range for all groups (45-55 years), was taken during the period from 20/1/2021 to 30/5/2021.

Methods

The CRP concentration was estimated using the VEDA.LAB devices according to the following method [13].

Estimation of D-dimer

Several kits made by Boditech Korea were used to assess the level of the dimer.

3. CBC (Complete blood count)

The complete blood count was measured using the swelap device, through the following steps:

1. We take (2 ml) of blood, then put the drawn blood into an anticoagulant tube (EDTA) Ethylene Diamine tetra acetic acid.
2. We put the tube on the shaker for 10 minutes, as this device mixes the components of the tube. The tube is placed in the complete blood image examination device (CBC), which contains three main solutions (diluent, cleaner, and Lyse), where the device gives a complete blood image.

4. Estimation of Metaloendopeptidase activity

The activity of the metalloendopeptidase enzyme was estimated according to the Kanazawa & Johnston method. The enzyme analyzes the casein substrate, and the absorbance of the resulting substance is measured at a wavelength of 275 nm. The activity was estimated as enzyme units per milliliter of blood serum. As for the enzymatic unit (U), it is the amount of enzyme that converts one micromole of the base substance into the resulting substance per minute per milliliter of blood serum [14].

5. Estimation of Interleukin-6 level

The concentration of interleukin-6 was estimated using ELISA technology using the double ELISA Sandwich antibody method, as the manufacturer's instructions were followed in all parts of the process, starting from the treatment of the attached reagents and solutions, preparing standard concentrations, testing steps, and reading the results. IL6 antibodyIL6-specific HRP-Conjugate reagent is added to each Microelisa strip plate well and incubated, after which standards or samples are added to the corresponding Microelisa strip plate pits and mixed with the designated antibodies, the liquid will turn blue after being thoroughly cleansed and added chromogen solutions A and B. The color changes to yellow after the Stop Solution is added, and the absorbance is then determined at a wavelength of 450 nm since the value of the absorbance intensity is related to the level of IL6 in the blood serum.

6. Statistical Analysis

At the probability level ($p \leq 0.01$), the statistical tool SPSS - Statistical Package for the Social Sciences was employed to compare patient groupings with a group of healthy individuals. Receiver Operating Characteristic analysis was used to its value for both ill and healthy groups in terms of (Sensitivity, Specificity), as well as (Curve).

Table 1: Shows the Mean ± S.D for all groups

Groups Variables	Mean ± SD		P-Value
	C	G1	
CRP(mg/L)	8.52±0.984	59.11±16.97	0.001***
D.dimer (ng/ml)	201.54±53.484	783.19±84.832	0.001***
WBC (109/l)	14.87±4.32	25.5± 9.23	0.001***
Lymph (%)	21.77±5.11	3.96±1.26	0.001***
IL-6 Pg/ml	4.96±1.26	15.74±3.11	0.001***
Metalloendopeptidase IU/L	45.28±13.28	77.26±24.12	0.001***
C: Control, G1: patients P≤0.001 = ***			

7. Evaluation of C - reactive protein in serum blood

In the above Table 1, standard deviation of the CRP levels the mean were (59.11 ±16.97) mg/L in G1 and (8.52 ±0.984) mg/L in C. According to the findings, CRP levels significantly increased in G1 compared to C (P≤ 0.001), according to Fig 1.

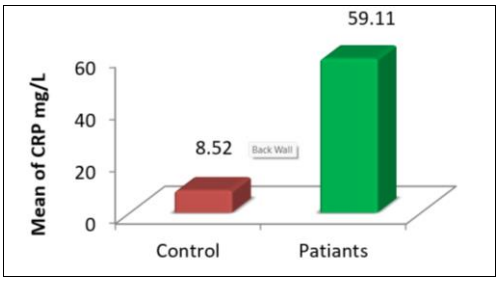

Fig 1: Level of CRP in the study group

This analysis findings corresponded with those of researchers [15], who found that the level of CRP increased in People infected with Corona. The usual concentration of CRP in the blood is less than (10 mg/liter) since this proportion rises fast within hours and reaches its peak within 48 hours of the disease's start and Coronavirus infection [16]. Several studies have recently found that CRP is related to severe dengue infection [17]. CRP levels can potentially indicate the extent of lung damage and the severity of the disease [18].

CRP levels increase as a consequence of viral infections being cleared out; the immune system then reacts by ramping up synthesis of various immunological components, including CRP. above the threshold cause organ failure in Covid-19 virus patients [19].

8. Estimation of D-dimer in plasma blood

The mean ± S.D of the D-dimer in G1 was (783.19± 84.832) mg/L and (210.54 ±53.484) mg/L in C, according to Table 1. CRP levels in G1 increased significantly (P≤0.001) when compared to C, as shown in Fig 2.

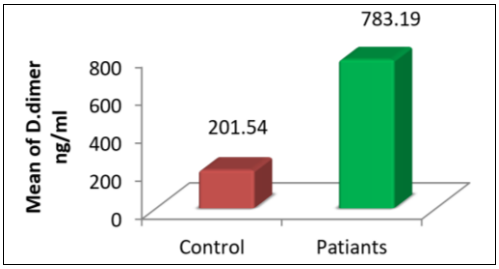

Fig 2: Level of D-dimer in the study group

This analysis findings are consistent with those of (Fu, 2020; Abas, 2021), who found that patients with COVID-19 had high D-dimer levels in their study. Dimer can be employed as a biomarker in Covid-19 patients because it has been linked to high levels of mortality in people with the disease. Dimer values and their outcomes (Franco, 2016). In order to take the necessary corrective action and lower the death rate, the dimer analysis can also be useful in monitoring the disease state of those infected with the emerging coronavirus and the transmission of the infected person from the dangerous intermediate stage to the very dangerous stage (Ahmed ani, 2019). Elevated D-dimer levels in a COVID-19 patient indicate a hypercoagulable state produced by the viral infection, which is generally accompanied by an intensive pro-inflammatory response and poor modulation of the anti-inflammatory response (Wong, 2017).

9. Estimation of WBC in blood

According to Table 1, the mean and standard deviation of the WBC in G1 were (25.5 ±9.23) 10 ^{9}/l and (14.87 ± 4.32) 10^{9}/l in C. As shown in Fig 3, the results showed that WBC levels rose in G1 when compared to C (P ≤0.01).

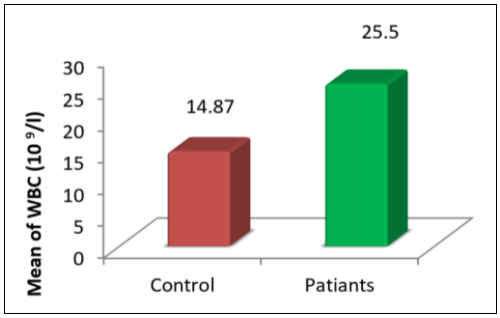

Fig 3: Level of WBC in the study gr

This analysis findings are consistent with those of (Pozdnyakova, 2021) and (Wang. *et al*., 2020), who found that patients with COVID-19 had elevated levels of white blood cells and confirmed that these values were common in patients and were associated with an increase in disease severity and in-hospital mortality.

According to certain research, the severity of the disease is correlated with an increase in the number of white blood cells (WBC) in more than 25% of severe cases [20]. Also, neutrophil counts increased in severe infections [21, 46].

And a decrease in platelets at the onset of infection [22]. It was also found that hemoglobin decreases with the progression of the disease [23].

10. Estimation of Lymphocyte in blood

The mean and standard deviation of the lymphocyte level in G1 were (3.96±1.26) % and (21.77±5.11) % in C, respectively, according to Table 1. The lymphocyte level in G1 was lower (P≤ 0.001) than in C, according to the data Fig 4.

The findings of the present investigation are consistent with those of [24, 25] who reported low lymphocyte counts in COVID-19 patients and confirmed in their study that a decline in lymphocyte numbers suggests the presence of impaired host response to disease control. According to a recent meta-analysis study, people with Covid-19 infections have fewer lymphocytes in 35% to 75% of cases. While they discovered that the number of injured people who passed away is higher (Lippi, 2020).

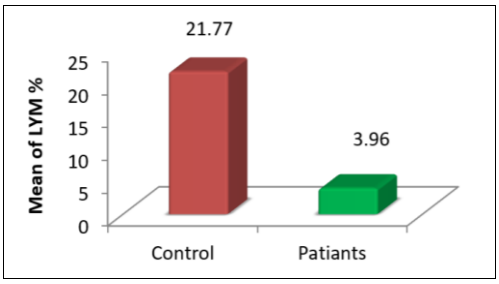

Fig 4: Level of Lymphocyte in the study group

The decrease in lymphocytes in people with COVID-19 varies geographically, for example, two studies published in Singapore and China for patients with the emerging coronavirus, found only a small percentage of patients included in the two studies had low lymphocyte counts [26, 27].

Patients with COVID-19 infection frequently have low levels of lymphopenia in their blood. It is thought that it stands for a virus-specific immune response. Lymphocytes are essential for sustaining the body's immune system and inflammatory response. Understanding the mechanism of reduced levels of lymphocytes in the blood is expected to provide an effective strategy for treating COVID-19 [21, 27].

11. Estimation of IL-6 in Serum blood

The mean and standard deviation of IL-6 levels in G1 were (15.74 ± 3.11) pg/ml, respectively, and (4.96 ± 1.26) pg/ml in C. According to the findings, IL-6 levels in G1 were higher (P≤ 0.001) than in C, as shown in Fig 5.

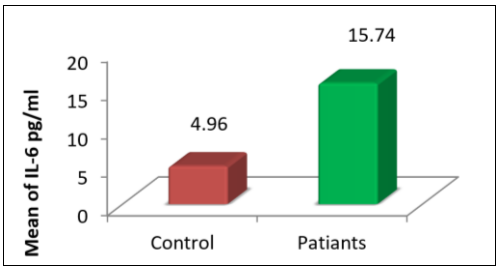

Fig 5: Level of IL-6 in the study group

The current study's findings were consistent with those of previous investigations [28, 29] who verified in their research that covid-19 patients had significantly higher levels of interleukin-6, which activated the host's dysregulated immune responses and may have contributed to the development of acute respiratory distress syndrome [30]. And this excessive humoral immune system activation, which is accompanied by a strong response from IL-6, may indicate that the pathophysiology of the complicated disease involves an irregular and excessive host inflammatory response [31, 42].

A cytokine storm, also known as cytokine release syndrome, could play important role in the pathogenesis of the COVID-19 pandemic, causing ARDS, multiorgan failure, and death. This is supported by elevated levels of inflammatory cytokines. One of the cytokines that have been repeatedly demonstrated to be important in COVID-19 is IL-6 [32, 33].

12. Estimation of Metaloendopeptidase in Serum blood

Table 1 showed Mean ± S.D of the Metaloendopeptidase was (77.26±24.12) IU/L in G1 and (45.28±13.28) IU / L in the C group. The results showed a rise (P≤0.001) in Metaloendopeptidase in G1 as compared with C group, Fig 6.

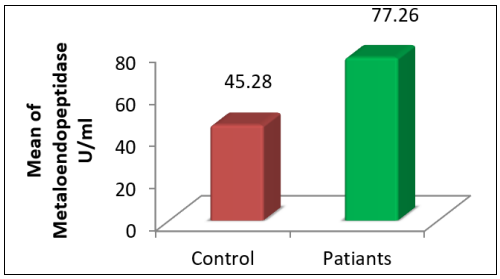

Fig 6: Level of Metaloendopeptidase in the study group

The link between enzyme activity and Covid-19 viral infection was not discussed in the literature., but [34, 45] indicated that infection with the Covid-19 virus shows a genetic expression of the enzyme in the cell wall between 12-24 hours from the onset of viral infection, and this explains the high effectiveness of the enzyme in patients.
Membrane metallopeptidase, also known as Neprilysin, is an extracellular enzyme bound to the cell membrane that can hydrolyze a range of peptides, including insulin, thus exacerbating the symptoms of diabetes when infected with a viral infection that activates its secretion. It may also target IL-6 and β- amyloids the enzyme has an important new regulatory role for insulin receptors in adipose tissue [35, 47].

13. ROC curve calculation

The ROC curve was calculated for the diagnostic and immunological variables for COVID-19 patients, represented by (C-reactive protein, D-dimer, and interleukin-6) in addition to the healthy group.

14. ROC curve for C-reactive protein in sera of all groups

The findings revealed that the area under the curve's (AUC) value was (1), the cutoff value was (6.75), and the sensitivity was (100), while the specificity was (93.33) at the probability level (<0.0001) for the two groups of patients and healthy ones, as in the Fig 7.

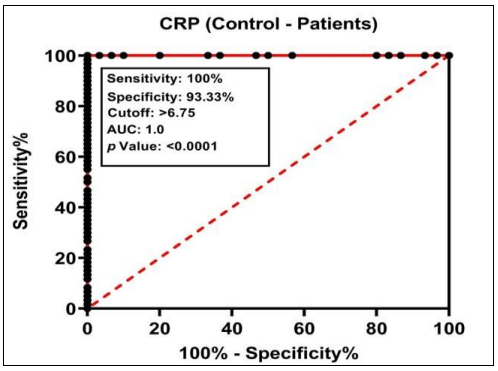

Fig 7: Analysis of the ROC curve for CRP in the study group

The results of the study agreed with the results of [36, 37] Who indicated that CRP levels can be used as a sensitive indicator of the development of COVID-19.

15. ROC curve for D. dimer in plasma of all groups

According to the findings, the area under the curve (AUC) value was (1), the cutoff value was (304.0), and the sensitivity was (100), while the specificity was (90.32) at the probability level (<0.0001) for the two groups of patients and healthy ones, as in the Fig 8.

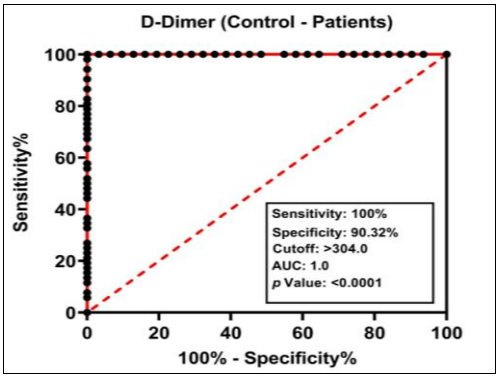

Fig 8: Analysis of the ROC curve for D.dimer in the study group

The study's findings agreed with those of [38, 39]. They demonstrated in their study that D.dimer is a useful predictive indicator for monitoring in-hospital mortality in COVID-19 patients at the viral infection stage. It also helps doctors to give the amount of treatment and provide intensive care urgently to those who need it most.

16. ROC curve for IL-6 in sera of all groups

The results showed that the value of the area under the curve (AUC) was (1) and (Cutoff) was (<5.409) and the sensitivity was (100), while the specificity was (88.89) at the probability level (<0.0001) for the two groups of patients and healthy ones, as in the Fig 9.

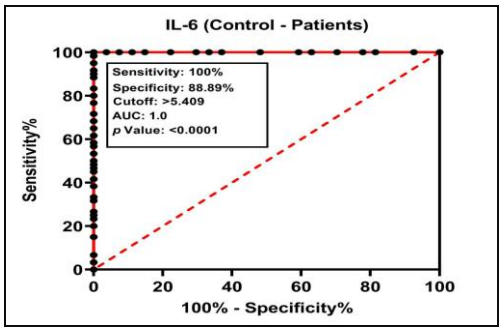

Fig 9: Analysis of the ROC curve for IL-6 in the study group

The current investigation's conclusions were consistent with those of [40, 41]. Researchers found that the level of IL-6 in the serum was closely associated with the severity of SARS COVID2 and that a significant increase in IL-6 could indicate that, the patient's condition is deteriorating and potentially life-threatening.

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