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Plasma D-Dimer Value Corrected with Some Physiological and Inflammatory Markers (C-Reactive Protein and Ferritin) in Iraqi Patients with COVID-19 Infection

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ABSTRACT

Since the beginning of the COVID-19 pandemic it has been observed that patients have elevated plasma levels of D-dimer and some inflammatory markers (ferritin, interleukine 6, C-reactive protein (CRP) or fibrinogen). Some studies point to the existence of a certain correlation between those markers and D-dimer. CRP is a protein discovered in the 1930s by Tillett and Francis and is an acute phase reactant. It is a pentameric protein which is synthesized by the liver under the action of cytokine interleukin 6 (IL-6). D-dimers are multiple peptide fragments produced as a result of degradation of crosslinked fibrin, mediated by plasmin. A total of 60 patients were recruited and categorized into :1- group1 (controls), 2- group 2 (COVID-19 patients). 5 ml of blood was obtained from each patient by vein puncture, using 5 ml disposable syringes, then centrifuged at 3000 rpm for 10 minutes to collect the serum. D- dimer, C-reactive protein were measured by using (Roche Diagnostics GmbH, Mannheim, Germany). At the same time, the Ferritin was assessed by using a miniVIDAS analyzer for the fluorescent enzymatic detection of β2-microglobulin (β2M) using the technique.

Enzyme Linked Fluorescent Assay (ELFA) (BioMerieux). Our results showed that there was a non- significant difference in the P-values between control and patients males and females. The Mean ± SE of age in control group was 45.90 ± 3.34, while the Mean ± SE of age in patients was 45.35 ± 2.52. There was a non- significant difference between the two groups, the Mean ± SE of CRP in control group was 5.04 ±0.81, while the Mean ± SE of CRP in COVID-19 patients was 37.16 ±3.24, there was a highly Significant differences between them (P≤0.01).

The CRP of COVID-19 patients were compared with those of control patients, the results shows significant increased CRP in covid-19 patients group as a compression with the control group, the findings of the study is similar to Jacob Lentner, etal findings. In response to infections, the liver synthesizes significant quantities of acute-phase proteins (APPs), such as CRP. This acute inflammatory protein is a highly sensitive biomarker for inflammation, tissue damage, and infection. It has been shown that CRP levels are correlated with levels of inflammation. CRP levels can promote phagocytosis and activate the complement system. In other words, CRP binds to microorganisms and promotes their removal through phagocytosis. D-dimers are one of the fragments produced when plasmin cleaves fibrin to break down clots. Our study showed that the serum D-dimer concentrations in patients significantly higher than those in control group which is similar to Mamta Soni, etal, 2020 findings.

Elevated D-dimer levels have emerged as a consistent finding in severely ill COVID-19 patients, Multiple studies have identified an association between higher D-dimer levels and an increased risk of mortality in the COVID-19 patient population.

Ferritin is an iron-storing protein; its serum level reflects the normal iron level and helps the diagnosis of iron deficiency anemia. Circulation ferritin level increases during viral infections and can be a marker of viral replication. Our study showed a significant increase in ferritin level in covid-19 patients group compared to control group, which consider similar to [] results.

Although the exact cause for elevated ferritin in COVID-19 infection is unknown, it could be influenced by cytokine release or cellular damage that results in the leakage of intracellular ferritin. It has been previously shown that ferritin is a direct indicator of cellular damage suggestive of an association between organ damage and ferritin production. This could later cause cell death, known as ferroptosis. It is suggested that inflammation associated with sepsis could alter iron metabolism and deficiency to facilitate the immune system, which could be an early sign of COVID.

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Keywords- D-dimer, C-reactive protein, ferritin.

I. INTRODUCTION

Since the beginning of the COVID-19 pandemic it has been observed that patients have elevated plasma levels of D-dimer and some inflammatory markers (ferritin, interleukine 6, C-reactive protein (CRP) or fibrinogen) (Tang *et al.*, 2020 ; Dujardin *et. al*., 2020). Some studies point to the existence of a certain correlation between those markers and D-dimer (Al-Samkari *et. al*.,2020).

CRP is a protein discovered in the 1930s by Tillett and Francis and is an acute phase reactant. It is a pentameric protein which is synthesized by the liver under the action of cytokine interleukin 6 (IL-6). A very high level of CRP >50 mg/dL is mostly associated with bacterial infections but elevated levels are also seen in injuries, cardiovascular processes and other inflammatory states. Elevated CRP levels not only suggest a pro-inflammatory state but also can be used as a prognostic marker for the underlying disease processes (Sproston *et al*., 2018)

D-dimers are multiple peptide fragments produced as a result of degradation of crosslinked fibrin, mediated by plasmin (Adam *et al*.,2009). The presence of D-dimers indicates the production and degradation of crosslinked fibrin, reflecting the coagulation and fibrinolysis processes occurring concomitantly. In healthy subjects, it is measurable in small amounts, because 2–3% of fibrinogen is converted to fibrin and enters the fibrinolytic pathway under normal physiological conditions (Thachil *et. al*., 2017). Any processes that involve production and breakdown of fibrin cause an elevation in D-dimer levels. These include acute venous thromboembolism (VTE), cancer, pregnancy, acute or chronic inflammatory states, acute infections and surgery. As it lacks specificity its role in the current scenario is mainly limited to rule out acute VTE. D-dimer levels vary among patients with confirmed VTE depending on clot burden, timing of measurement, and initiation of treatment (Linkins and Takach, 2017). The body contains iron in the form of ferritin, which is an intracellular protein composed of 24 subunits circling an iron core containing 4000–4500 iron atoms (Domellof *et al*.,2002). Ferritin is an intracellular protein composed of 24 subunits surrounding an iron core containing 4000–4500 iron atoms. Ferritin is a mediator for immune dysregulation, especially in hyper-ferritinemia, with direct immune suppressive and pro-inflammatory effects that cause cytokine storms (Abbaspour *et al*., 2014) . The cytokine storm syndrome causes dangerous outcomes in covid-19 disease and the prevalence of the symptoms is depending on the cytokine cloud syndrome (Jin *et al*., 2020) . Serum ferritin levels are closely linked to the incidence of covid-19 disease (Liu et al., 2020). Manipulation of serum ferritin levels may be used to reduce nutritional iron (Ju and Ha, 2016).

Aim of the study:

To evaluate the value of D- dimer, C- reactive protein and ferritin in COVID- 19 patients.

II. MATERIALS AND METHODS

2.1 Subjects and study design

A total of 60 patients were recruited and categorized into :

1- group1 (controls)

2- group 2 (COVID-19 patients)

2.2. Methods:

Blood Sample Collection:

The following biochemical investgations have been studied for the estimation of D- dimer, C-reactive protein and ferritin. 5 ml of blood was obtained from each patient by vein puncture, using 5 ml disposable syringes, then centrifuged at 3000 rpm for 10 minutes to collect the serum. D- dimer, C-reactive protein were measured by using (Roche Diagnostics GmbH, Mannheim, Germany). At the same time, the Ferritin was assessed by using a miniVIDAS analyzer for the fluorescent enzymatic detection of β2-microglobulin (β2M) using the technique Enzyme Linked Fluorescent Assay (ELFA) (BioMerieux).

2. 3. Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. T-test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

III. RESULTS

Table 3.1 showed that there was a non-significant difference in the P-values between control and patients males and females.

Table 3.1: Distribution of study samples according to Sex in different Groups

Group	No	Male No. (%)	Female No. (%)	P-value
Control	10	4 (40.00%)	6 (60.00%)	0.184 NS
Patients: COVID-19	40	20	20	1.00 NS

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		(50.00%)	(50.00%)	
P-value	---	0.207 NS	0.207 NS	---
NS: Non-Significantly.				

In the table 3.2, The Mean ± SE of age in control group was 45.90 ± 3.34, while the Mean ± SE of age in patients was 45.35 ± 2.52. There was a non- significant difference between the two groups.

Table 3.2: Comparison in age between different groups

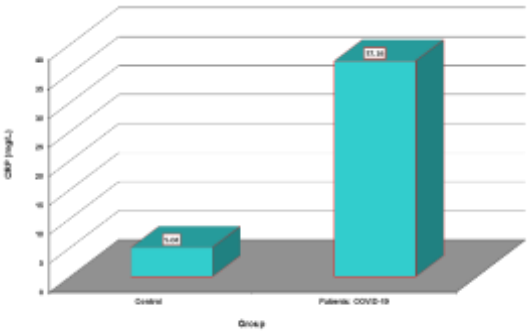
Group	No	Mean ± SE Age (year)
Control	10	45.90 ± 3.34
Patients: COVID-19	40	45.35 ± 2.52
T-test	---	10.758 NS
P-value	---	0.918
NS: Non-Significantly.		

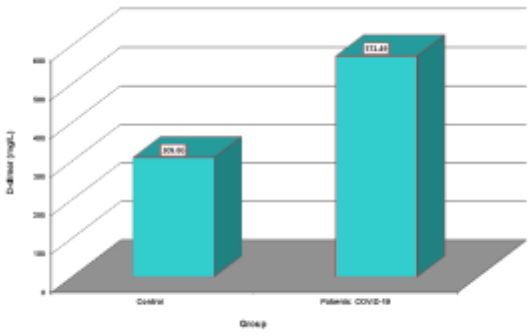
Table 3.3 showed that the Mean ± SE of CRP in control group was 5.04 ±0.81, while the Mean ± SE of CRP in COVID-19 patients was 37.16 ±3.24, there was a highly Significant differences between them (P≤0.01).

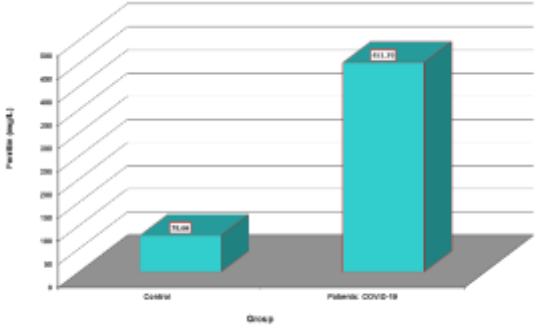
Table 3.3: Comparison between difference groups in CRP, D-dimer and Ferritin

Group	Mean ± SE		
	CRP (mg/L)	D-dimer (mg/L)	Ferritin (mg/L)
Control	5.04 ±0.81 b	309.06 ±40.76 b	78.66 ±17.10 b
Patients: COVID-19	37.16 ±3.24 a	572.49 ±40.09 a	451.79 ±38.61 a
T-test	13.159 **	167.27 **	157.38 **
P-value	0.0001	0.0027	0.0001
Means having with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).			

Significant (P≤0.05)* Highly Significant (P≤0.01) **NS: Non Significnt







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IV. DISCUSSION

C-reactive protein is well established as a marker of systemic inflammation and severe infection. As an acute-phase reactant, CRP binds to phosphocholine in pathogens and membranes of host cells, and acts as an opsonin to enhance phagocytosis and facilitate clearance (Nathaniel R Smilowitz, etal, 2021).

In the current study, the CRP of COVID-19 patients were compared with those of control patients, the results shows significant increased CRP in covid-19 patients group as a compression with the control group, the findings of the study is similar to (Jacob Lentner, etal, 2021).

In response to infections, the liver synthesizes significant quantities of acute-phase proteins (APPs), such as CRP (Khalil R. H., etal). This acute inflammatory protein is a highly sensitive biomarker for inflammation, tissue damage, and infection (Sproston N. R., etal). It has been shown that CRP levels are correlated with levels of inflammation. CRP levels can promote phagocytosis and activate the complement system (Gershov D., Kim S.etal). In other words, CRP binds to microorganisms and promotes their removal through phagocytosis (Povoa P., Pereira J., etal).

D-dimers are one of the fragments produced when plasmin cleaves fibrin to break down clots (Hayıroğlu Mİ, etal, 2020). Our study showed that the serum D-dimer concentrations in patients significantly higher than those in control group which is similar to (Mamta Soni, etal, 2020) findings.

Elevated D-dimer levels have emerged as a consistent finding in severely ill COVID-19 patients, Multiple studies have identified an association between higher D-dimer levels and an increased risk of mortality in the COVID-19 patient population (Hayıroğlu Mİ, etal, 2020).

Ferritin is an iron-storing protein; its serum level reflects the normal iron level and helps the diagnosis of iron deficiency anemia. Circulation ferritin level increases during viral infections and can be a marker of viral replication (Baraboutis IG, etal, 2020). Our study showed a significant increase in ferritin level in covid-19 patients group compared to control group, which consider similar to [Shilia Jacob Kurian, etal.2023] results.

although the exact cause for elevated ferritin in COVID-19 infection is unknown, it could be influenced by cytokine release or cellular damage that results in the leakage of intracellular ferritin (M. Pujani, etal .2021).

It has been previously shown that ferritin is a direct indicator of cellular damage suggestive of an association between organ damage and ferritin production. This could later cause cell death, known as ferroptosis. It is suggested that inflammation associated with sepsis could alter iron metabolism and deficiency to facilitate the immune system, which could be an early sign of COVID (F.T. Bozkurt, etal 2021).

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