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Evaluation the role of CRP, D-dimer and osteopontin as biomarkers in assessing the severity of COVID-19 patients with pre-existing diabetes

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***Abstract*---**COVID-19 is a new coronavirus that has the potential to be deadly. The World Health Organization (WHO) has declared the COVID-19 outbreak to be an international public health emergency. According to new evidence, comorbid diabetes in COVID-19 patients is linked to disease progression and even death. Aim of study is to evaluate the role of serum osteopontin, CRP and D-Dimer activity in the severity of covid19 and possibility of using these biomarker as predictors for mortality of covid19 in diabetic patients. This is a cross sectional, hospital based study. This study was carried out from 1st December 2021 to 30th January 2022 and included 60 Patients admitted to COVID19 isolation centers in Alshefa-14 and Kirkuk general hospital (30 COVID-19 without D.M and 30 COVID-19 with D.M) and 30 control samples. All data were collected through a face-to-face interview by a questionnaire. The current study showed a significant increase in all the measured parameters in patients with positive COVID-19 when compared to control group with no apparent clinical diseases. D-dimer increased in patients by a mean of (3215 ng/l, SD ± 50) when compared to control group (218 ng/L SD ± 27.3) with (p-value <0.05). And when this level is compared between the patient groups (severe non-diabetic, sever diabetic and mild), it's noted that its level mostly increased in the severe non-diabetic (3912 ng/l, SD ± 49.99) and sever diabetic (1602 ng/l, SD ± 1602) patient group when it is compared to the control and mild group with (P value < 0.05). CRP increased in the severe non-diabetic (111 mg/dl, SD ± 18.30) and sever diabetic (162 mg/dl, SD ± 23.0) patient groups more

than both mild (15 mg/dl, SD ± 16.6) patient groups and control group (5 mg/dl, SD ± 1.22) with (p-value <0.05). Osteopontin concentration is increased in the patients sever diabetic (29.31ng/ml, SD ± 1.64) and sever non-diabetic group (21.27 ng/ml, SD ± 1.96) when it's compared with control group (11.59 ng/ml, SD ± 3.21) with (p-value <0.05). And its level is the highest in Sever patient group when compared with the both mild (diabetic and non-diabetic) patient groups (9.79 ng/ml, SD ± 3.0) and control group (10.1 ng/ml, SD ± 3.21) with (p-value <0.05). Because higher levels of D-dimer, CRP and Osteopontin were linked to a worse prognosis and outcome, these measures can be used as predictors for illness progression or follow-up after therapy.

***Keywords*---**COVID-19, osteopontin, CRP, D-dimer, diabetes.

Introduction

On December 31, 2019, the Chinese Ministry of Health alerted the World Health Organization (WHO) of several infected individuals in Wuhan, Hubei, central China, with an unclear cause (Lu et al., 2020). On January 7, the World Health Organization identified a new coronavirus, 2019-nCoV, in a patient's throat swab sample (Hui et al., 2020). The World Health Organization eventually subtitled this pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Patients at high risk of severe COVID-19 or mortality have had several attributes, including advanced age and male sex, as well as underlying health issues such as cardiovascular disease (CVD), obesity, and/or type 1 or type 2 diabetes mellitus (T1DM) or type 2 diabetic mellitus (T2DM) (GorbalenyaAE, 2020). According to a few early studies, patients with COVID-19 admitted to critical care units ICUs frequently had underlying CVD and diabetes mellitus (Burki, 2020). The global SARS-CoV-2 epidemic has immediate implications for the treatment of common metabolic diseases such as type 2 diabetes (T2D). Obesity is also emerging as a significant comorbidity for SARS-CoV-2 illness severity. Obesity is known to increase the risk of influenza complications, and in the context of SARS-CoV-2, obesity is emerging as an important comorbidity for disease severity (Critchley et al., 2018). In coronavirus disease 2019, a systemic inflammatory response is observed (COVID-19). In bacterial or viral infections, elevated serum levels of C-reactive protein (CRP) D-Dimer and Osteopontin are markers of systemic inflammation, are associated with severe disease (Pavlou et al., 2018). Diabetes is among the primary comorbidities related with infection severity in the COVID-19 pandemic. Other comorbidities include being older, male, and having underlying medical illnesses such chronic lung disease, cardiovascular disease, and hypertension. Late diabetes complications, such as diabetic renal disease and ischemic heart disease, can make people frailer and worsen the severity of COVID-19 illness, leading to kidney or heart failure (Richardson et al., 2020).

Methodology

Patient and methods

This is a cross sectional, hospital based study. The agreement of attendance to Al-shefa-14 hospital, Kirkuk general hospital that approved by Kirkuk health directorate, to collect the samples from the patients. This study was carried out from 1st December 2021 to 30th January. Patients admitted to COVID19 isolation centers in previously mentioned hospitals. An interview was carried out with these patients using questionnaire form designed by the investigator including their name, age, etc.

The study involved 60 patients with positive RT-PCR for COVID-19 and 30 control samples. They are classified into three groups according to CT scan, SPO2 levels and clinical assessment by physicians:

- Group 1: COVID-19 without Diabetes (15 sever, 15 mild).
- Group 2: COVID -19 with Diabetes (15 sever, 15 mild).
- Group 3: control (30 sample).

Methods

Blood samples were obtained from the patients and controls. Blood samples of 5 ml were taken from antecubital vein puncture. The blood sample obtained from each subject was transferred into gel tube for separation of serum. Then blood in the gel tubes were then allowed to clot at room temperature (25 ˚C) for 30 minutes .After that centrifugation was done at (4000) rpm for 10 minutes to separate the serum. The serum of each patient and control was divided and stored in to 3 small tubes and immediately 3 test was done then the rest stored at -80˚C in Cryo-Refrigerator until the time of analysis to avoid thawing and refreezing. Thawing of the samples was allowed to take place at 25˚C before conducting the assay.

Table 1
shows instruments used in this research

Instrument name	Source
ELISA washer and reader	Biotek USA
Spectrophotometer (APEL-PD-303)	Japan
Incubator (ALFA)	Turkey
Centrifuge (80-1 ELECTRONIC)	China
Gel Tube (serum tube)	China
Sodium citrate tube (whole blood)	China
Deep Freezer (HAMCO 47 SERIES)	India
Syringe	China
Eppendorf	Denmark
Tips(blue 1ml,yellow 100M)	
Cotton	
Gloves	
Adhesive tape to protect the vein puncture site after blood drawing	

Statistical analysis

The result were presented in tables and statistical significances were assessed by the SSPS version 27 using one way Anova T-test to compare between patient groups and control with P-value <0.05 considered significant, then post hoc analysis is done to compare between patient groups.

Results

Table 2
Distribution of Studied Groups According to SPO2% Levels

Groups	Sub-Groups	N	Mean %	St.Dev	95% CI	P-Value
COVID-19	Mild	15	97.00 a	± 1.13	93.08, 100.91	> 0.05
	Sever	15	69.97 c	± 11.00	65.75, 73.58	< 0.05
COVID-19 D.M	Mild	15	95.00 b	± 1.10	92.00, 100.01	< 0.05
	Sever	15	77.60 c	± 10.64	74.83, 80.37	< 0.05
Control		30	99.47 a	± 0.50	96.69, 102.23	

N=number, St.Dev= Standard Deviation, CI= Confidence interval D.M= Diabetes mellitus. a: the mean value statistically not significant with control. b and c: the mean value statistically significant with control and with each other.

Test results in Table (2) show that there is significant deference according to SPO_2 level between deferent groups is lowest in the studied patient groups (Sever non-diabetic and sever with D.M) with mean of SPO_2% (73%, SD ± 10.82) compared to the control group (99.47%, SD ± 0.5) with P-Value (< 0.05).

Table 3
Distribution of Studied Groups According to Chest Involvement by CT scan

Groups	Sub-Groups	N	Mean %	St.Dev	95% CI	P-Value
COVID-19	Mild	15	5.00 a	± 0.00	0.11, 10.11	> 0.05
	Sever	15	32.00 b	± 6.12	26.89, 37.11	< 0.05
COVID-19 D.M	Mild	15	5.00 a	± 0.00	0.12, 10.10	> 0.05
	Sever	15	35.00 b	± 3.00	31.38, 38.62	< 0.05
Control		30	0.00 a	± 0.00	3.61, 3.61	

N=number, St.Dev= Standard Deviation, CI= Confidence interval D.M= Diabetes mellitus. a: the mean value statistically not significant with control. b and c: the mean value statistically significant with control and with each other.

Test results in Table (4-2) show that there is significant deference according to CT levels between deferent groups is higher in the studied patient groups (Sever non-diabetic and sever with D.M) with mean of chest involvement by CT (33%, SD ± 4.65) compared to the control group (0.00%, SD± 0.00) with P-Value (< 0.05).

Table 4
distribution of studied groups according D-Dimer levels

Groups	Sub-Groups	N	Mean ng/L	St.Dev	95% CI	P-Value
COVID-19	Mild	15	205 a	± 23.9	1039.1, 1449.1	> 0.05
	Sever	15	3912 b	± 49.6	2668, 5156	< 0.05
COVID-19 D.M	Mild	15	207 a	± 24.2	1049.1, 1469.1	> 0.05
	Sever	15	2602 c	± 44.8	723, 2482	< 0.05
Control		30	218 a	± 27.3	661.3, 1098.1	

N=number, St.Dev= Standard Deviation, CI= confidence interval.
a: the mean value statistically not significant with control.
b and c: the mean value statistically significant with control.

Test result in table (2) shows that there is significant deference's according to D-Dimer levels between deferent groups and it is higher in both studied patient groups (Sever) with mean of (3257 ng/L, SD ± 47.23) in compare to the control group (218 ng/L, SD ± 27.3) with P-Value (< 0.05).

Table 5
distribution of studied groups according to CRP levels

Groups	Sub Groups	N	Mean mg/dl	St.Dev	95% CI	P-Value
COVID-19	Mild	15	10 a	± 3.79	18.35, 40.43	> 0.05
	Sever	15	111 b	± 18.30	131.7, 192.5	< 0.05
COVID With D.M	Mild	15	20 c	± 9.79	15.35, 51.43	< 0.05
	Sever	15	162 d	± 23.00	90.3, 133.3	< 0.05
Control		30	5 a	± 1.22	17.46, 25.53	

N=number, St.Dev= Standard Deviation, CI= confidence interval.
a: the mean value statistically not significant with control.
b, c, d: the mean value statistically significant with control.

Test result in table (6) shows that there is significant deference's according to CRP levels between deferent groups and it is higher in both studied patient groups

(Sever) with mean of (136.5 mg/dl, SD ± 20.65) in compare with control group (5mg/dl, SD ± 1.22) with P-Value (< 0.05).

Table 6
Distribution of studied groups according to Osteopontin levels

Groups	Sub-Groups	N	Mean ng/ml	St.Dev	95% CI	P-Value
COVID-19	Mild	15	9.29 a	± 2.69	15.56, 28.99	> 0.05
	Sever	15	21.27 b	± 1.96	-0.42, 13.01	< 0.05
COVID-19	Mild	15	10.29 a	± 3.01	17.06, 25.09	> 0.05
D.M	Sever	15	29.31 c	± 1.64	2.06, 11.56	< 0.05
Control		30	11.59 a	± 3.21	6.84, 16.33	

N=number, St.Dev= Standard Deviation, CI= confidence interval.
a: the mean value statistically not significant with control.
b and c: the mean value statistically significant with control.

Test result in table (4) shows that there is significant deference's according to osteopontin levels between deferent groups is higher in both sever patient groups with mean of (25.29 ng/ml, SD ± 1.8) and highest concentration in sever diabetes group (29.31 ng/ml, SD ± 1.64) compared to the control group (11.59 ng/ml, SD ± 3.21) with P-Value (< 0.05).

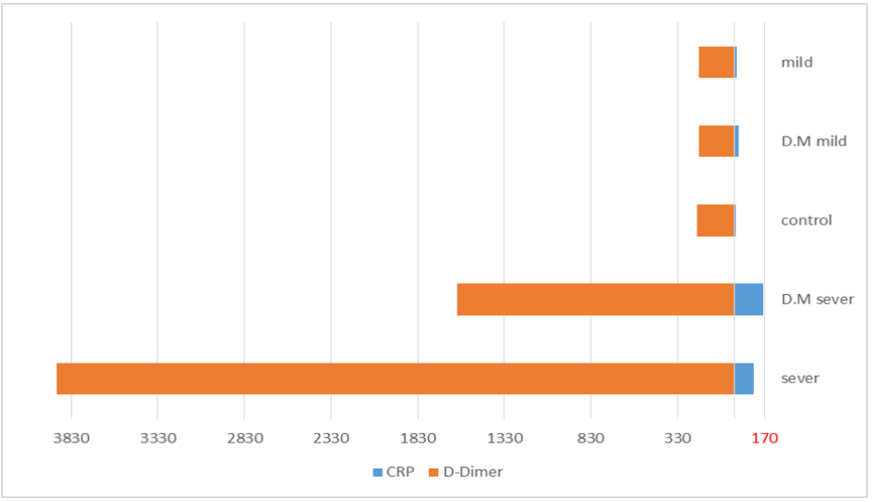

Figure 1. Correlation of CRP and D-Dimer Levels between Study Groups.

According to the results in (figure 1) there is positive correlation between D-dimer and CRP, which means whenever there is D-dimer level increase, CRP level will be increased also. High concentration found in sever groups both with and without diabetic with highest concentration of CRP observed in Sever non-diabetic group (162 mg/dl) and highest concentration of D-Dimer observed in sever without diabetes group (3912 ng/L).

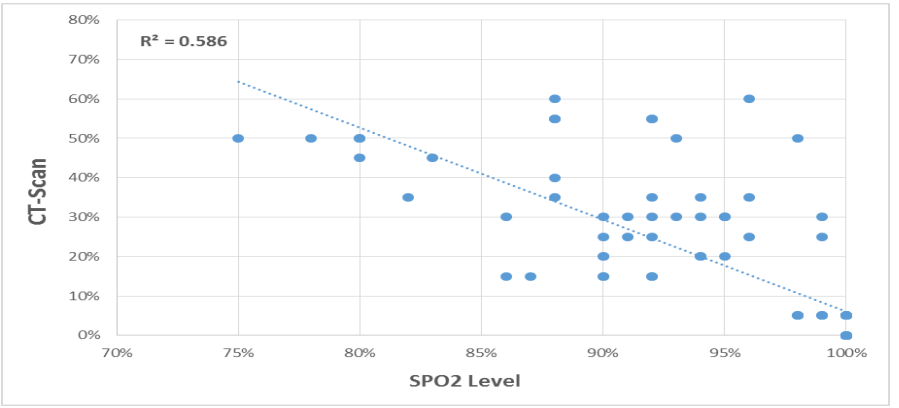

Figure 2. Correlation of SPO2 Levels with CT scan in Study Groups

The study showed that there was a negative correlation between increased level of SPO2 saturation and chest involvement by CT scan (R2=0.586). Increased chest involvement in disease causes decrease in SPO2 saturation.

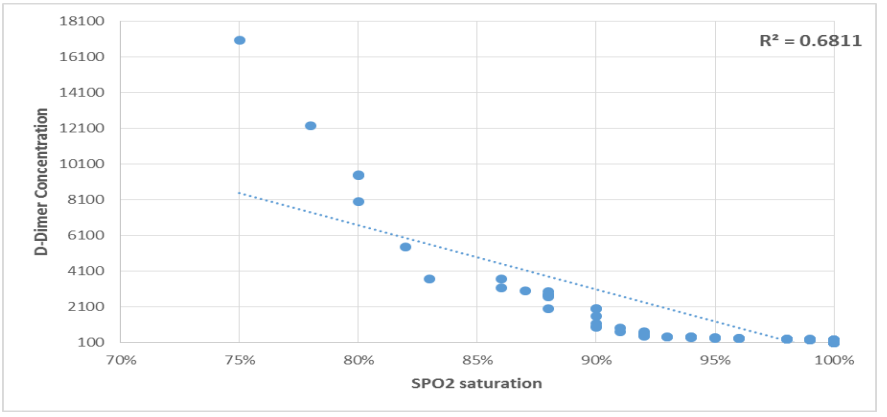

Figure 3. Correlation of SPO2 Levels with D-Dimer in Patient Groups

The study showed that there is a negative correlation between level of SPO2 saturation and D-Dimer concentration (R2=0.6811). The more D-Dimer level increases the more SPO2 saturation decreases.

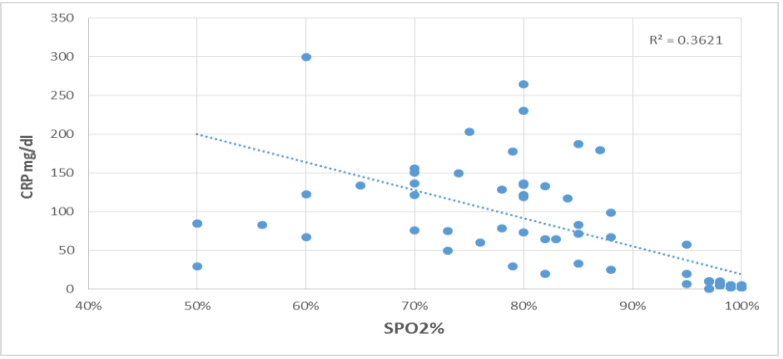

Figure 4. Correlation of SPO2 Levels with CRP Concentration in Patient Groups.

The study showed that there is negative correlation between SPO2 saturation point and CRP concentration (R2=0.362), the more CRP concentration increases the more SPO2 level decreases.

Discussion

Study groups where classified into mild and sever according to SPO2 saturation and chest involvement by CT scan with (SPO2% > 96%) is considered mild and (SPO2 < 90% is considered sever) (Shenoy et al., 2020). Chest involvement (< 5 %) is considered mild and (> 20% is considered severe) (Schoen et al., 2019). Test result in table (2) shows that there is significant deference's according to D-Dimer levels between deferent groups is higher in the studied patient groups (Sever non-diabetic and sever Diabetic) compared to the control group (P-Value < 0.05). This deference in results is compatible with studies in which shows that D-dimer has previously been used as a hypercoagulability marker (Yang et al., 2017). Coagulation abnormalities with marked elevations in D-dimer levels in COVID-19 patients have been observed in studies. Recent research has linked D-dimer levels greater than 2000 ng/mL to an increase in COVID-19 severity and fatality (L. Zhang et al., 2020). Severe hypoxia in ARDS patients can activate the extrinsic coagulation pathway and increase blood viscosity, resulting in a hypercoagulable state in COVID-19 infection (Gupta et al., 2019).

Diabetes patients had severe disease, with higher D-dimer levels in the severe group than in sever diabetic group (P < 0.01). Hyperglycemia can cause endothelial dysfunction and inflammation, which can result in thrombus formation (Domingueti et al., 2016). As a result, severe acute respiratory infection is more likely to result in coagulopathy and a poor outcome. The results of the test in table (3) shows that there is a significant difference in CRP levels between (Sever non-diabetic and sever Diabetic) groups in compare to the control group (P-Value> 0.05). CRP is higher in sever group than Diabetic group (P < 0.05). The current study provided a positive correlation between plasma CRP levels and disease severity. CRP concentrations increased significantly in the diabetic and severe groups, respectively, (p < 0.05). Furthermore, higher CRP levels in severe-diabetic SARS-CoV-2 pneumonia patients were found to have a higher selectivity than lower CRP levels in those with a mild condition (W. Zhang et al., 2020).

In severe COVID-19 patients, elevated CRP levels may be linked to an overproduction of inflammatory cytokines. Cytokines fight microbes, but when the immune system becomes overactive, it can cause lung tissue damage. Thus, in COVID-19 patients, inflammatory cytokines and tissue destruction induce CRP production (Ali, 2020). Test result in table (4) shows that there is significant deference's according to osteopontin levels between deferent groups is higher in the studied patient group (sever non-diabetic and sever diabetic) compared to the control group (P-Value < 0.05). OPN levels are related to disease severity, with higher levels in more severe disease groups compared to moderate disease group. The sever group had the highest OPN levels. These findings are consistent with the findings of (Varım et al., 2021) who discovered that OPN was related to disease severity in a cohort of 84 adult COVID-19 patients.

OPN is a multifunctional and widely distributed glycoprotein. OPN is highly stable in blood and exists in low concentrations in healthy people, making it an appealing candidate biomarker. The feasibility of point-of-care measurement would increase the clinical utility of OPN. While OPN is known to be released in a variety of tissues and cell types, OPN is physiologically involved in both inflammation and immune responses. Previous research has shown that OPN is released by immune cells and that it plays a role in early T lymphocyte activation, as a chemotactic factor for neutrophils, mast cells, and macrophages, and in immunoglobulin production (Inoue & Shinohara, 2011; Lanteri et al., 2012; Lund et al., 2009).

Conclusion

The level of all parameters measured in our study (D-dimer, CRP and osteopontin) are higher in patients with COVID-19 when compared to the control group with no apparent clinical condition. D-dimer can be used as a predictor of COVID-19 severity and the development of thromboembolic events in such individuals. Measured plasma CRP level significantly increased in the severe patient group then in Diabetic group when it's compared to mild and control groups, so it can be used as a marker to assess the severity and progression of COVID-19 alone or with D-Dimer. Measured serum Osteopontin levels significantly increased in the severe and diabetic groups when it's compared to the control and group.

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