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Study of Plasminogen activator inhibitor-1 (PAI-1) in hospitalized COVID-19 patients

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ABSTRACT

Background: There is an evidence that COVID-19 individuals have impaired fibrinolysis, which may increase their thrombotic risk further. Hepatocytes and endothelial cells are responsible for the synthesis of plasminogen activator inhibitor-1 (PAI-1). It is the primary inhibitor of tissue-type plasminogen activator (t-PA) and has a crucial role in fibrinolysis control. The purpose of our research was to measure the plasma levels of PAI-1 in Patients with COVID-19 who were hospitalized.

Methods: In the current research, 40 Patients with COVID-19 and 40 healthy participants served as controls. Enzyme-linked immune-sorbent assay (ELISA) was used to measure plasma PAI-1 levels.

Results: There was a considerable elevation in WBCs count and segmented WBCs in cases compared to control with significant decrease in lymphocytes in cases compared to control group. PAI-1, CRP, LDH, Ferritin and D-Dimer were remarkably raised in patients compared to control group.There was positive significant correlation between PAI-1 levels and urea, creatinine, AST, LDH, ferritin, D-Dimer and CRP. PAI-1 showed 93% sensitivity and 87% specificity at cut off 5.5 ng/ml in discriminating patients from control group**.**

Conclusions: Plasma concentrations of PAI-1, a measure of fibrinolytic homeostasis, were higher in ICU patients with COVID-19. This research also showed that PAI-1 may be added to the list of biomarkers used to define Patients with COVID-19.

Keywords:COVID-19,Biomarkers,PAI-1, Fibrinolytic, Homeostasis.

INTRODUCTION

Several acute uncommon respiratory disorders emerged in December 2019 in Wuhan, China, and quickly spread to neighbouring cities. The culprit was swiftly identified as a new coronavirus. This new coronavirus was discovered given the name respiratory distress syndromecoronavirus-2 (SARS-CoV-2) due to its 70% similarity to SARS, which is a single-stranded RNA coronavirus with a positive sense that predominantly infects human cells[1].

ACE2 is overexpressed in lung alveolar cells, vascular endothelial cells and cardiac myocytes among others. SARS-CoV-2 is mostly spread by respiratory tract invasion and inhalation. This virus may live for 24–72 hours on transmission-friendly surfaces[2]. Infection with SARS-CoV-2 may cause mild to severe failure in respiration and multiple organ failure. Asymptomatic people may have pulmonary ground glass opacification seen on computed tomography (CT) scan [1].

Hypercoagulability is associated with COVID-19, which manifests as an enhanced risk of venous thromboembolic events and microvascular target-organ damage. The relationship between significant inflammation and coagulation is crucial for estimation the prognosis of severe Patients with COVID-19[3].

Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor with a single chain glycoprotein (or serpins). It contains 379 amino acids and a 50 kDa molecular mass. PAI-1 is the major suppressor of tissue-type plasminogen activators (t-PA) and urinary-type plasminogen activators (u-PA). Megakaryocytes, endothelial cells, monocytes, macrophages, smooth muscle cells, adipocytes, cardiac myocytes and fibroblasts are the types that make PAI-1.[4].PAI-1, as a possible biomarker, a key regulator of fibrinolysis was discovered. PAI-1 reduces plasminogen activator, a necessary enzyme in the conversion of plasminogen to plasmin. This causes intravascular coagulation, hypoperfusion, and organ failure by inhibiting fibrinolysis[5].

COVID-19 infected patients may have poor fibrinolysis, which may increase their thrombotic risk. This has been shown by dramatically diminished clot lysis at 30 minutes using thromboelastography (TEG) in individuals with severe COVID-19 infection. Moreover, Patients with COVID-19 had significantly increased PAI-1 levels that are associated with diminished fibrinolytic ability [6]. This study aimed to evaluate the plasma levels of PAI-1 in COVID-19 hospitalized patients.

PATIENTS AND METHODS

The current research was done on 80 patients who were divided into two groups: group1 included 40 Patients with COVID-19 ranging in age from 21 to 63 years, and group2 contained 40 healthy persons ranging in age from 20 to 60 years who acted as the control group. Patients were recruited from COVID-19 ICU Hospitals at Tanta University.

Inclusion criteria:

Established diagnosis of COVID-19 as indicated by a positive nasopharyngeal viral PCR for SARS-CoV2, and all control subjects were healthy, non-medicated, and exhibited no signs of pathological conditions.

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Exclusion criteria: Age <18 years old, severe uncontrolled hypertension (SBP>200 consistently >12 hours, Platelets <50,000), cerebrovascular accident history, internal significant bleeding, recent intraspinal or intracranial surgery or trauma, as well as severe liver failure and metastatic cancer.

All patients underwent a comprehensive history, clinical examination, standard laboratory investigations (CBC, Liver function tests, Kidney function test, LDH level, CRP, ferritin, and D-Dimer), specialised laboratory investigations (Enzyme-linked immune-sorbent assay (ELISA) was used to measure plasma PAI-1 levels.

Measurement of PAI-1 using ELISA technique:

A blank well containing neither sample nor standard was built, along with standard wells holding 50µl of standard and test wells containing 40µl of sample. 10µl of Biotin-PAI-1 antibody was added to the test wells, and 50µl of Streptavidin-HRP was applied to both the standard and test wells. The membrane was then sealed, gently shook, and stored at 37 °C for one hour. The fluid was removed by carefully removing the membrane from the container. In each well, 50µl of solution A chromogen then mixed gently with 50µl of the added solution B chromogen and incubated for 10 minutes at 37 °C without light. The reaction was halted by adding 50µl of stop solution (the blue soon became yellow), and 15 minutes after adding the stop solution, the optical density (OD) was evaluated at 450 nm wavelength.

Calculation of results: The standard curve was generated by plotting each standard's mean absorbance against its concentration graphed on a standard two-dimensional bar chart, with concentration along the X axis and absorbance along the Y axis. Using this standard curve, the sample concentrations were determined directly.

Statistical analysis

SPSS V.22 was used for statistical analysis (IBM Inc., Chicago, IL, USA). Quantitative data were given as mean and standard deviation (SD) and compared using the paired Student's t-test. Qualitative data were analysed by number and percentage (%) and Chi-square test was used for comparing qualitative variables. Linear correlation coefficient test was used to compare two quantitative variables. ROC curve was also performed. A two tailed P value ≤ 0.05 was considered significant.

Ethical approval: This research was authorised by the Ethical Committee of the Faculty of Medicine, Tanta University. Informed permission was obtained from each participant.This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

RESULTS

Regarding CBC, there was a substantial rise in WBCs count (P= 0.045) and segmented WBCs (P= 0.001) but a considerable decrease in lymphocytes (P= 0.001) in cases vs controls (**Table 1).**

Table (1): CBC values in all subjects

	Patients (n=40)	**Control (n=40)**	**P value**
Hb (g/dl)	12.46 ± 1.78	12.29 ± 1.39	0.640
Platelets (10^3/µl)	221.3 ± 111.25	204.52 ± 41.99	0.375
WBCS (10^3/µl)	10.58 ± 6.4	8.4 ± 2.17	0.045*
Segmented (%)	80.73 ± 6.92	68.93 ± 8.33	0.001*
Lymphocytes (%)	14.08 ± 6.37	26.10 ± 7.68	0.001*

Data were presented as mean ± SD.Hb: haemoglobin, WBCs: white blood cells.

The urea and creatinine concentrations of patients were substantially greater than those of healthy controls (P = 0.001). ALT and AST in patients' group were considerably higher than in control groups (P = 0.001). Albumin and total protein levels in sick groups were considerably lower than in control groups (P = 0.001). Insignificant statistical difference variation was recorded between the patient and controls' total bilirubin levels (P = 0.173) (**Table2).,**

Table 2: Routine laboratory data in all subjects

	Patients (n=40)	**Control (n=40)**	**P value**
Urea (mg/dl)	74.25 ± 56.70	38.08 ± 10.59	0.001*
Creatinine (mg/dl)	1.45 ± 1.16	0.92 ± 0.19	0.001*
ALT (IU/L)	86.45±84.96	30.75 ± 7.94	0.001*
AST (IU/L)	72.68±73.24	31.10 ± 6.68	0.001*
S. Albumin (g/dl)	3.23 ± 0.57	3.95 ± 0.42	0.001*
Total protein (g/dl)	5.75 ± 0.82	6.78 ± 0.61	0.001*
Total bilirubin (mg/dl)	0.99 ± 1.29	0.71 ± 0.26	0.173

Data were presented as mean ± SD. ALT: Alanine transaminase, AST: Aspartate transaminase,

Patients exhibited significantly higher levels of the inflammatory markers CRP, lactate dehydrogenase (LDH), Ferritin (P value= 0.001), and D-Dimer (P value= 0.009) than controls**(Table 3).**

Table 3: the inflammatory markers in all subjects

	Patients (n=40)	Control (n=40)	P value
CRP (mg/l)	44.68 ± 37.98	5.13 ± 3.66	0.001*
LDH (U/L)	363.03 ± 177.65	165.63 ± 25.52	0.001*
Ferritin (ng/ml)	1036.80 ± 1241.71	82.58 ± 35.37	0.001*
D- Dimer (ng/ml)	1.67 ± 3.39	0.23 ± 0.13	0.009*

Data were presented as mean ± SD. CRP: C-reactive protein, LDH: Lactate dehydrogenase.

In addition, when COVID-19 severity increased, PAI-1 levels in patients' group increased markedly (P = 0.001)**(Table 4).**

Table 4: PAI-1 level in all subjects

	Patients (n=40)	Control (n=40)	P value
PAI-1 (ng/ml)	12.58 ± 6.39	3.45 ± 1.14	0.001*

Data were presented as mean ± SD.PAI-1: Plasminogen activator inhibitor-1.

Positive and significant correlations were between PAI-1 levels and urea, creatinine, AST, LDH, ferritin, D-Dimer, and with CRP (r = 0.298, P = 0.062)**(Table 5).**

Table 5: Correlation between PAI-1 level and other variables in studied groups

	PAI 1	
	R	P
Hb	-0.060	0.713
PLTs	0.032	0.843
WBCs	0.280	0.080
Urea	0.326	0.040*
Creatinine	0.320	0.043*
ALT	0.011	0.946
AST	0.393	0.012*
Total bilirubin	0.236	0.142
Albumin	-0.254	0.113
Total protein	-0.022	0.894
LDH	0.465	0.003*
Ferritin	0.569	0.001*
D-Dimer	0.748	0.001*
CRP	0.298	0.062

* statistically significant p<0.05.

The ROC analysis of PAI-1 revealed that the optimal cut off level for differentiating patients from the control group was 5.5 ng/ml with an area under the curve (AUC) of 0.986, yielding a sensitivity of 93%, specificity of 87%, PPV of 88%, NPV of 92%, and 90% accuracy (**Figure 1).**

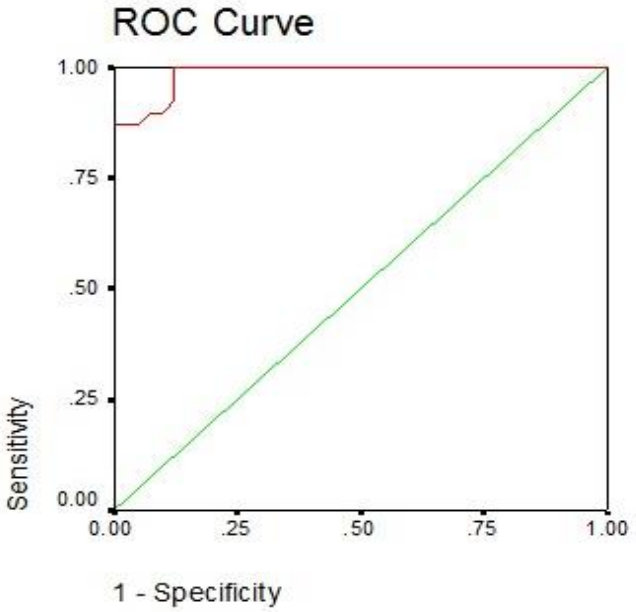

Figure 1: ROC curve of PAI -1

DISCUSSION

In the recent study, the incidence of COVID-19 was 1.5 times greater in men than in women across the examined groups.This is in agreement with **Tadiri *et al.*** [1] and **Jin *et al.*** [2]They discovered that men are more prone than women to develop severe instances of COVID-19.

In the current study, WBCs count (leucocytosis), Neutrophilia, and Lymphopenia in Patients with COVID-19 exhibited a significant statistical variation (P = 0.001) based on laboratory data from the analysed groups. This is in line with **Huang *et al.*** [3]who demonstrated that COVID-19 severe patients had considerable leucocytosis, neutrophilia, and lymphopenia. Also the study by **Sayad *et al.*** [4] also concluded a significant relation between leucocytosis and the rate of mortality in COVID-19 patients. In contrast to the results of the present work **Liu *et al.*** [5] who stated that leukopenia was seen among Patients with COVID-19.

There was no significant variation in haemoglobin level between the patient and control group. In contrast to the results of the present work **Algassim *et al.*** [6]who discovered that a low haemoglobin level correlates with a more severe illness course and a greater fatality rate.

Insignificant difference in Platelet count was observed between the patient and controls. Nevertheless, **Xu *et al.*** [7] who found that COVID-19 cases have thrombocytopenia.

There were substantial discrepancies in urea and creatinine levels across patient groups as COVID-19

severity rose. This agrees with **Xia *et al.*** [8] who discovered that COVID-19 individuals had substantially elevated levels of urea and creatinine. There was a notable variation in ALT and AST results across patient group as disease severity progressed (p = 0.001). This is in accordance with **Hwaiz *et al.*** [9] who found that ALT and AST values were elevated in COVID-19 subjects.

The severity of COVID-19 was correlated with a significant difference in blood albumin and total protein levels across patient groups (p = 0.001).This is in agreement with **Huang *et al.*** [3] who found that COVID-19 patients had significant decrease in levels of serum albumin and total protein.

CRP is a known marker of inflammation and infection since CRP levels increase much higher than other acute phase reactants during acute inflammation. The CRP test has been used to indicate infection, systemic inflammation, or sepsis [10].There was a significant difference in CRP levels in patient groups as their levels increased with severity of COVID-19 (pvalue =0.001). This agrees with **Chen *et al.*** [11] who found that subjects with COVID-19 had significant increase in levels of CRP.

LDH enzyme is crucial in the glycolysis and a cellular enzyme that is present in most organs. Its level increases in inflammation and cellular destruction. Viral mRNA clearance ratio was strongly correlated with LDH concentrations.[12].There was a significant increase in LDH levels in patient groups as their levels increased with severity of COVID-19 (pvalue =0.001).This agrees with **Henry *et al.*** [13] who found that patients with COVID-19 had significant increase in levels of LDH.

Serum ferritin is a protein that stores iron and is regarded as one of the most essential acute phase reactants. Through direct immune-suppressive and proinflammatory actions, elevated ferritin levels may lead to a cytokine storm. In severe instances of COVID-19, ferritin levels rise, which is a critical immune response mediator [14].There was also a remarked increase in Ferritin levels in patients as their levels increased with severity of COVID-19 (pvalue =0.001).This agrees with **Kaushal *et al.*** [15] who found that subjects with COVID-19 had significant increase in levels of Ferritin.

Levels of D-Dimer also rose dramatically in patients' group as their levels were increased with COVID-19 severity (pvalue =0.001).This agrees with **Yao *et al.*** [16] who found that Patients with COVID-19 had significant increase in levels of D-Dimer. Unlike, **Zuo *et al.*** [17] found that there was no relation between D-Dimer and PAI-1.

Although the research only comprised a limited sample of participants, there was a statistically significant increase in PAI-1 levels as COVID-19 severity increased (p = 0.001). PAI-1 appears to be evidence of endothelial impairment in COVID-19.This is in agreement with **Cabrera-Garcia *et al.*** [18] who found that PAI-1 levels were substantially higher in patients with COVID-19. In individuals with COVID-19, their research revealed that PAI-1 is a potent marker of coaglo-fibrinolytic diseases.

Our study showed insignificant relation between serum PAI-1in the studied groups and their age and gender. **Hoshino *et al*.** [19] showed insignificant correlationbetween serum PAI-1 levels and the age. Contrasting, **Lorente *et al*.** [20] who observed significant increased PAI-1 levels in elderly patients and **Koyama *et al*.** [21] results where the PAI-1 was slightly higher in men contrasted to women.

In the present study, PAI-1 levels had significant positive correlation with urea, creatinine, AST, LDH, Ferritin, D-Dimer (P. value =0.001). No significant correlation was found between PAI-1 and Hb level, platelets count, WBCs count, ALT, total bilirubin, serum albumin, total protein and CRP.This is in agreement with **Panigada *et al*.** [22] who found that there were no significant association between platelet count and PAI-1. Also, **Hoshino *et al.*** [19] found that there were no relation between WBCs count and PAI-1.

However, there were limitations to this research, such as its limited sample size and the fact that it was conducted at a single institution. Moreover, the study lacked patients follow up.

CONCLUSIONS

PAI-1 plasma levels, a measure of fibrinolytic homeostasis, were higher in ICU patients with COVID-19. This research also showed that PAI-1 may be added to the list of biomarkers used to diagnose COVID-19.

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