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Research Article
D-Dimer Level Among COVID-19 Patients as Biological Mediator for Hyper Coagulation State

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Abstract

Background and Objective: Viremia due to SARS-CoV-2 lead variety of biochemical change in the human body, which play a crucial role in the activation of the coagulation cascade causing thrombotic complications and coagulopathies. The study aimed to ascertain the D-dimer level as a biological mediator in COVID-19 patients in Khartoum state and compare the results to the control group. **Materials and Methods:** A cross-sectional study was conducted during the period of August to December, 2021, including 50 healthy patients and 50 COVID-19 patients, blood samples were collected from study groups for measurement of D-dimer level using an I Chroma device. Statistical analysis was conducted using SSPS version 21. **Results:** This study revealed a statistically increased D-dimer level among COVID-19 patients compared with the control group (2000-10000 vs. up to 500 ng mL^{-1}), respectively. **Conclusion:** Viremia induced by COVID-19 infection can cause a high D-dimer level which can lead to thrombosis event or bleeding tendency.

Key words: D-dimer, SARS-CoV-2, viremia, hyper coagulopathy, coagulation cascade, thromboembolism, thrombosis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coronavirus disease 2019 ((COVID-19) Coronaviruses are enveloped, single-stranded RNA viruses) has affected people all over the world and resulted in the vast majority of deaths since the pandemic's spread in December, 2019 in Wuhan, China. The COVID-related fatality rate is closely linked with hypercoagulability and an elevated chance of Venous Thromboembolism (VTE) events, which can ultimately have led to thrombo-inflammation in serious conditions[1,2]. As a consequence, coagulation biomarkers may imply the severity of disease and death rates, as well as assist in patient triage, treatment interventions and prognosis strategic planning. D-dimer is a fibrin degradation product that plays a mechanistic role in COVID-19 thrombo-inflammation[3,4].

As noted by numerous studies, SARS-CoV-2 stimulates an inflammatory process in the lower airways, eventually leading to pulmonary infection[5,6]. Viral particles penetrate the respiratory mucosa, provoking immune responses and a "pro-inflammatory cytokine storm" which is mainly correlated to COVID-19 patients' critical illness. The COVID-19 manifestations varied from SARS-CoV, MERS-CoV and influenza virus in concepts of viral tropism. Even before adaptive immune response, the innate immune system serves to decelerate viral infection[7]. The majority of patients have lymphocytopenia as well as normal or low WBCs counts. Even though the granulocytes count, D-dimer, creatinine and urea level, are markedly increased in critically injured patients, the lymphocyte count is limited. The immune system responds to viral' molecular structures' (i.e., double-stranded RNA), whereas, adaptive immunity eradicates viral-infected cells via cellular and humoral cells that generate viral-specific immunoglobulins[8,9].

D-dimer is among the protein fragments formed whenever a thrombus diffuses throughout the bloodstream. It is regularly impossible to detect or noticeable at very small concentrations except if the body is forming and disintegrating blood clots, in which case its level in the blood can dramatically emerge and increased. The D-dimer assessment is used to diagnose procoagulant (thrombotic) occurrences and to enable the diagnosis of diseases associated with venous thromboembolism. It is conducted whenever a patient has manifestations of a thrombus or an event that affects unnecessary clots in the blood, such as in venous thrombosis (DVT), Pulmonary Embolism (PE), etc.[10]. Numerous studies have linked elevated D-dimer (predominance up to 46.4%) to acute exacerbation and negative events of COVID-19. Patients with D-dimer levels higher than 1000 ng mL^{-1} have a 20-fold greater risk of mortality than those with reduced D-dimer amounts[11,12]. As a consequence, extremely high D-dimer levels are a prognostic marker for VTE in COVID-19 patients and pharmacotherapy antithrombotic doses focused on D-dimer lowering are more effective for patients than preventative doses[13].

Therefore, the prevalence rate of symptomless deep vein thrombosis in patients with COVID-19 pneumonia outbreak of novel coronavirus disease globally should raise alarms because patients are at hazard of thrombosis and bleeding. The current study attempted to determine the shifts in D-dimer thresholds among Sudanese patients infected with COVID-19 in Khartoum state, to evaluate their efficacy in thrombosis prevention.

MATERIALS AND METHODS

Hospitalized SARS-CoV-2 confirmed infected patients and necessitating intubation and mechanical ventilation were enrolled in this cross-sectional study for measurement of D dimer level, as well as healthy Sudanese subjects recruited as a control group. The study was conducted in Khartoum State for a period from July, October, 2021. All real-time PCR positive COVID-19 patients were included in their study regardless of their age and gender. Subjects with any encountered diseases that may affect D-dimer level (such as malaria, liver disease, renal disease, all types of cancer, etc.) were excluded from the study.

Ethical consideration: The Ethical Committee of the Academic staff of Clinical Laboratory Sciences at Al-Zaiem Al-Ezhari University in Khartoum, Sudan, approved this study. Before participating, each subject provided consent forms.

Sample size and sample collection: Of a total of 100 subjects, 50 were SARS-CoV-2 patients and 50 were healthy control patients. The venous blood sample was collected using sterile, dry, plastic syringes with no long time tourniquet applied to make the veins more prominent. The puncture sites were sterilized with 70% ethanol, a total of 5 mL of blood in 3.2% sodium citrate from each volunteer. Blood samples were then centrifuged at 1500 g for 15 min to yield platelet-free plasma that was ready for use[14].

Method of D dimer estimation: D-dimer level was tested with immunofluorescence method by using a rapid quantitative kit dedicated to i-chroma II instrument *in vitro* diagnostic medical device. By using D-dimer detection buffer and disposable D-dimer cartilage. Plasma was mixed with fluorescently labelled probe antibody before being loaded,

the fluorescence concentration can be determined after 12 min of incubation by checking the test cylinder and transferring it to the level of D-dimer in a laser fluorescent detector. The automated standard analysis was used to measure the comparability of the designed assay[15].

D-dimer test procedure: Insert I chroma II D-dimer specific ships to the device. About 10 uL of platelet-free plasma was added to the detection buffer kit and mixed 10 times. Then take 75 uL from the mixture and add it to D-dimer cartilage. Incubate the cartridge for 12 min in 25°C. Then insert the cartridge into the device to read the result, which is in the normal range (up to 500 ng mL^{-1}).

Statistical analysis: Data were analyzed by using the SPSS computer program version 20. Descriptive statistics were determined (Minimum, Maximum, Mean (±SD). Also, an independent T-test was applied to compare two parametric data to obtain a p-value ($p<0.05$ was considered statistically significant).

RESULTS

The present study enrolled 50 confirmed SARS-CoV-2 screened by real-time PCR, compared to 50 control participants. The majority of patients 33 (66%) were males and their age group ranged between 30-75 years old, with no statistically significant differences between patients and control, shown in Table 1.

Table 2 illustrated the means differences in D dimer level among COVID-19 patients and control groups, where D-dimer level was statistically significant elevated (4352.2±310.3 vs. 419.3±201.2) among SARS CoV-2 patients and control, respectively.

A statistically significant difference in D-dimer means level correlates with advanced age and then more disease complications (R-value and p-value 0.273 and 0.001), respectively. No statistically significant differences between D dimer mean levels and gender. All data were displayed in Table 3 and 4. The level of D-dimer was positively correlated with age as shown in Fig. 1.

Table 1: Demographic data of study participants

	Patients n = 50 (%)	Control n = 50 (%)
Gender		
Male	33 (66%)	26 (52%)
Female	17 (34%)	24 (48%)
Age group/mean SD	55±15.95	
35-50 years old	21 (42%)	12 (24%)
50-65 years old	19 (38%)	25 (50%)
65-90 years old	10 (20%)	13 (26%)

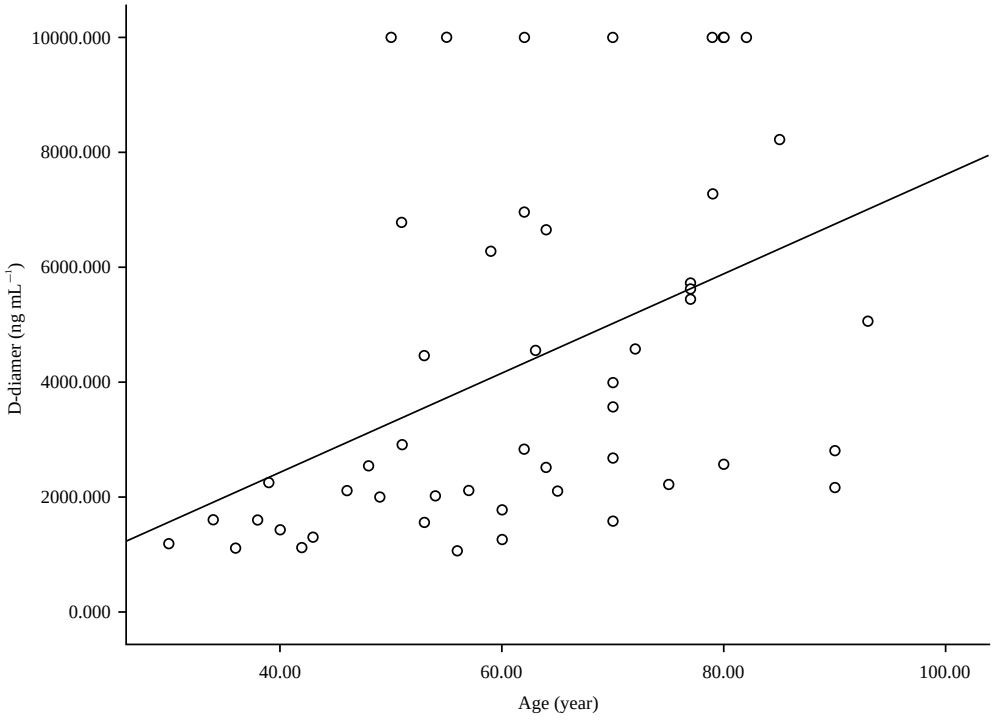

Fig. 1: Level of D-dimer was positively correlated with age

Table 2: Comparison of mean level of D-dimer between COVID-19 patients and healthy participants

		N	Mean	SD	p-value
D-dimer (ng mL^{-1})	Patients Mean±SD	50	4352.2	3103.2	0.0001
	Control Mean±SD	50	419.3	201.2	

Table 3: Correlation of D-dimer level to age in COVID-19 patients

	Age groups	N	Mean	SD	p-value	Pearson correlation
Age (year) and D-dimer (ng mL^{-1})	35-50 years old	21	4112.7	1201.6	0.001	0.444*
	50-65 years old	19	4260.2	1590.2		
	65-90 years old	10	4415.1	1032.5		

*Correlation is significant at a 0.01 level

Table 4: Comparison of D-dimer level within gender in COVID -19 patients

	Gender	N	Mean	SD	p-value
Age (year) and D-dimer (ng mL^{-1})	Male	33	4262.9	2.86	0.78
	Female	17	4526.0	3.60	

DISCUSSION

Coronavirus disease enhances the variety of biochemical changes in the human body. Many studies reported an association of COVID-19 infection with increased D-dimer level, which can lead to many disturbances in the human body, the most common impairments of D-dimer level are clots that can end in thrombotic episodes and increase morbidity and mortality. So this study was applied to ensure the benefit of this simple and critical test in the routine prognosis of COVID-19 among Sudanese people, because of ethnic diversity and different environments. The study aimed to determine the D-dimer level in COVID-19 patients and compare the results to the control group.

In the present study, the majority of COVID-19 participants were males (66%) which agreed with the study[16], which demonstrated that the incidence of COVID-19 is higher in males than that in females. This was due to dissimilar innate immunity, steroid hormones and factor related to sex chromosomes. As well as age group, the majority of patients were 35-50 years old, which is attributed to the reason that this age group is more vulnerable to the virus because many of them work in the community, this same finding noted as evidenced by other age groups who sit at home[17]. Concerning the advanced age group, 19 (38%) of COVID-19 patients were advanced age group Numerous previous COVID-19 research indicated that the 50+ inhabitants were more susceptible to infection than other age groups due to impaired immune systems and predominate health problems. Current results were inconsistent with numerous studies and observations that demonstrated that severe and deteriorated cases were in advanced age groups and above 50 years old[11,17,18].

This study showed that there is a significant increase in the mean level of D-dimer in COVID-19 patients compared to the control group ($p = 0.0001$). These results showed an agreement with the finding of Yu *et al.*[19], who found that the high D-dimer level was independently associated with COVID-19 than among control. As well as Crawford *et al.*[20] noted that all patients were admitted with COVID-19 and radiological findings of (Pulmonary Embolism) PE had plasma D-dimer levels of 0.05 g mL^{-1} or higher, according to this diagnostic study. These results can be linked to the danger of high ability of formation of thrombosis in COVID-19 patients. Following to study carried out by Zhan *et al.*[21] this study showed a significant increase in the mean level of D-dimer (p-value) when compared to the control group. D-dimer is well-known for its diagnostic character for discovering thrombotic event responses as one member of FDPs major fragments as clot signalling pathway in normal value to control the strength of thrombi degradation event.

The causes of the increased D-dimer levels are only partially explained. D-dimers have been shown to form throughout fibrin breakdown and serve as an indicator of fibrinolytic interaction. In serious patients or patients with sepsis, an association between pro-inflammatory cytokines and markers of coagulation cascade stimulation, such as D-dimer, has been evidenced[22,23]. Also, there is an indication that in inflammatory conditions, the alveolar hemostatic stability shifts toward a thrombogenic overrepresentation. Furthermore, in severely ill patients, pro-inflammatory cytokines may be involved in endothelial injury, as well as initiating coagulation and preventing fibrinolysis[24,25].

The study also noted a non-significant difference in the mean level of D-dimer in COVID-19 patients according to gender ($p>0.05$). The statistical quality analysis showed a positive correlation of D-dimer level with age in COVID-19 disease patients ($R = 0.4$) with a significant moderate correlation ($p = 0.001$). Which demonstrates the effect of an immunocompromised state and exhausted immune system due to advanced age on the devastating spread of virus and server inflammatory reaction that enhance coagulation factors and then increase D dimer production[17].

Even so, another issue that must not be neglected is that patients with COVID-19 have significantly greater D-dimer levels once their CRP concentration is lower than in CAP patients. This strongly indicates that moreover to inflammatory response, other factors come into play in the stimulation of the coagulation cascade in COVID-19 patients. Gralinski *et al.*[26] reviewed the pathogenesis of COVID-19 and identified an innovative host pathway involved in SARS's steady progress in a previous study. Their results indicated that urokinase pathway downregulation throughout SARS-coronavirus infection contributed significantly to more serious respiratory pathology and significant changes in systemic hemostatic stability.

As a result, all of the data suggested that D-dimer expression was strongly associated with the inflammatory process and generally tend to stabilize as the inflammatory process settled down in the vast majority of patients, emphasizing that inflammation is indeed one of the reasons that influence coagulation stimulation in patients with COVID-19 infection.

This study showed some limitations; first the small sample size and patients were recruited regardless of their clinical manifestation and COVID-19 patients are not classified based on disease severity, they were selected at random based on having COVID-19 disease only. Second, the study ignored the inflammatory mediators, as well as other coagulation profiles and CBC. Another study is required to compare the level of D-dimer according to the severity of the disease. Further study regarding other haematological and coagulation profiles is crucial to be done. Sophisticated and more research (hormonal and chromosomal studies) are needed to determine the association of disease with males rather than females.

CONCLUSION

This study concluded that, patients with COVID-19 had a marked increase in D-dimer level, which was correlated with old age and male was more affected by the disease than female. The COVID-19 patients should be followed and monitored by measuring D-dimer level as a sensitive biomarker for COVID-19 to improve the outcome of the disease.

SIGNIFICANCE STATEMENT

The value of D-dimer is a precise and reliable prognostic marker for estimating fatalities in COVID-19 patients, as 1.5 g mL^{-1} is considered the best possible threshold value of enrollment D-dimer for predicting death rates in COVID-19 patients.

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