Measurement of Plasma Fibrinogen Levels among HIV infected patients: A critical Bio-Marker for Coagulation Dysfunctions

Abstract:

Background: The literature stated that Human immunodeficiency virus (HIV) infection led to activation of coagulation, and habitually linked with an augmented risk of venous and arterial thrombosis. So the purpose of the study was to determine the plasma fibrinogen level in Sudanese HIV-infected patients. **Material and methods: A** total of one hundred participants were recruited, and classified into two groups; the case group include (50) HIV patients, and the control group enrolled (50) healthy individuals. Three ml of blood was collected. Fresh Poor Plasma was prepared from citrated venous blood by centrifuged for 15 minutes at 3000 pm. Fibrinogen levels were measured by an automated coagulation analyzer (Thrombolyzer XRC Germany). Data were collected using a directly structured questionnaire. Data were analyzed using SPSS Version 21. **Results:** The present study showed that the mean of plasma fibrinogen levels was statistically significantly higher in HIV infection in comparison with those normal healthy control (470.50 ±67.75 vs 214.75±21.25 with P-value 0.00). there was a significantly decreased level of PT, and PTT among the HIV group comparing with the control (9.575±0.64, and 22.39±4.94) VS (12.483±0.72, and 30.78±3.55) consequently, (P-value ≤0.001). fibrinogen levels were significantly increased with the progression of HIV disease (469.84 ±67.15, 472.74 ±87.75, 478.47 ±61.92) in stage I, stage II, stage III respectively. **Conclusions:** An HIV-infected patient had elevated plasma fibrinogen levels, as well as other coagulation dysfunctions.

Keywords: HIV, CD4, Fibrinogen, Coagulation.

Introduction:

Infection with the Human Immunodeficiency Virus (HIV) induces a gradual worsening of the immune system due to a decrease in the amount of CD4+, and helper T cells in circulation, the immune system has been compromised. [1]. HIV-associated thrombus formation had been ascertained during the HIV interval and is well documented in the literature. [2]. Numerous hemostatic malformations in HIV patients have been established, enabling mechanisms for hypercoagulability and an elevated chance of thrombosis. [3]. Infection with HIV causes systemic inflammatory disease with prominent hematological disorders. Such abnormalities increased in the last stage of disease, and are caused by a variety of factors, including immune-

mediated cell destruction, direct cytopathic consequences of the virus, secondary to potential pathogens and malignancies, and drug toxicity [4, 5].

The pathophysiology of HIV proposed that it enhanced microbial product and migration through intestinal mucosa as a result of persistent destruction to lymphatic tissue mucosa outcomes in the stimulation of monocyte, representation of tissue factor, and pathogenic hypercoagulability. [6]. Increased activation of platelets activation may even play an important role in hypercoagulation in HIV-positive patients, though the exact pathophysiology of HIV-related changes in platelet function is widely undefined. [7, 8]. Megakaryocytes have already been found to contain CD4 receptors on their coats, and then both megakaryocytes and platelets have been found to have the cytokine (CXC motif) receptor on their surfaces, making them vulnerable to HIV infection [9]. Platelets have been proved in vitro to incorporate particles of HIV, and virus-infected platelets have been demonstrated to produce activating signs [10]. The present study was aimed at the measurement of fibrinogen levels and other hematological parameters among Sudanese HIV patients.

Materials and methods:

An analytical case-control study was carried out during the study period from September to December 2018. Totally of 100 participants were recruited for the study. 50 subjects were HIV known patients, diagnosed by (ELSIA and PCR) technique, among them 25 (50%) were males and 25 (50%) were females: who were fellow the clinic at Omdurman Teaching Hospital, Khartoum/Omdurman during the study period and designated as the case group. Further 50 were healthy volunteers 26 (52%) were males and 24 (48%) were females, designated as a normal control group (Age and sex were matched between case and control group). Under the full aseptic technique, a total of 3 mL of venous blood was drawn. from all participants in 3.2% anticoagulant tri-sodium citrate containers in a 9 to 1 ratio, then Platelet Poor Plasma (PPP) was instantly processed by centrifugation at 3000 rpm for 15 min. An automated coagulation analyzer was used to determine plasma fibrinogen concentration (Thrombolyzer XRC Germany). Every HIV patient had: liver diseases, inflammatory, cancer, under heparin or warfarin therapy, and coagulopathy disorders were excluded. Pregnant women were excluded from the study in both study groups.

Data collection and analysis:

The data was collected using a directly structured questionnaire and analyzed by computer software SPSS Version 21. The parameters were compared in mean and Stander division using the T-test. The P-value was set a significant when is less than 0.05.

Ethical approval

This study was approved by the Faculty of Medical Laboratory Science, Al Neelain University institutional review board. Before samples were gathered all participants gave their consent; the information was taken kept very confidentially.

Result:

Among a total of one hundred subjects participates in the present study for measurement of plasma fibrinogen their age range between 20-50 years old, the mean ages of HIV patients group were 35.5±1.34 SD years old, and the mean age of normal control was 37.1±0.91 SD, with equal gender distribution; no statistically significant difference reported. The majority of HIV patients were in stage II, and infection duration from 2-4 years old (64%, and 56%) respectively. All data are illustrated in table 1.

Table 2 displays the mean level of Fibrinogen levels and other coagulation profiles among HIV patients and the control group. Findings revealed that the mean and stander deviation of plasma fibrinogen levels (mg/dl) was statistically significantly higher in the case group when compared with those normal healthy control group (470.50 ±67.75 vs 214.75±21.25 with a P-value of 0.001), nevertheless, there was the significantly decreased level of PT, and PTT among HIV group comparing with control (9.575±0.64, and 22.39±4.94) VS (12.483±0.72, and 30.78±3.55) consequently, (P-value ≤0.001). the platelets count was significantly decreased among HIV patients comparing with control (189.78±83, and 295.33±63).

Table 3 shows the mean level of fibrinogen and other coagulation factors to different HIV stages, our findings documented that fibrinogen levels were significantly increased with the progression of HIV disease (469.84 ±67.15, 472.74 ±87.75, 478.47 ±61.92) in stage I, stage II, stage III respectively.

Table 1: Baseline data of study subjects

	Patients n=50 (%)	**Control n=50 (%)**	**P value**

Gender			
Male	25 (50%)	26 (52%)	0.473
Female	25 (50%)	24 (48%)	
Age			
20-30 years old	15 (30%)	24 (48%)	
31-40 years old	21 (42%)	10 (20%)	0.582
41-50 years old	14 (28%)	16 (32%)	
HIV Stage			
Stage I	16 (32%)	-	
Stage II	32 (64%)	-	-
Stage III	2 (4%)	-	
Period of infection			
2-4 Years	28 (56%)	-	
4-6 Years	20 (40%)	-	-
≥ 6 Years	2 (4%)	-	
Total	50 (100%)	50 (100%)	

Table 2: Mean level of Fibrinogen levels and other coagulation profiles among HIV patients and control group.

Parameters	Case (n=50) Mean ±SD	Control (n=50) Mean ±SD	P-value
HBG	13.462±0.791	14.867±0.851	0.001
WBCS	4.321±1.21	6.972±1.75	0.001
Platelets	189.78±83	295.33±63	0.000
PT	9.575±0.64	12.483±0.72	0.002
PTT	22.39±4.94	30.78±3.55	0.005
Fibrinogen levels	470.50 ±67.75	214.75±21.25	0.001

- A P-value less than 0.05 is considered significant

Table 3: Comparison of Fibrinogen levels and other coagulation profiles among HIV patients

Parameters	HIV Stage			P-value
	Stage 1	Stage II	Stage III	
WBCS	5.024±0.21	3.711±0.84	2.537±1.21	0.023
Platelets	202.18±67	194.32±59	186.89±78	0.054
PT	10.446±0.59	11.958±0.341	12.325±0.322	0.005
PTT	22.39±3.86	23.57±3.23	25.14±1.87	0.041
Fibrinogen levels	469.84 ±67.15	472.74 ±87.75	478.47 ±61.92	0.001

Discussion:

Human immunodeficiency virus (HIV) plays a pivotal part in coagulation system activation and increases the risk of arterial and venous thrombosis that leads to atherosclerosis [11, 12]. For decades, hemostatic alterations in coagulation factor concentrations and state of hypercoagulation have been identified in HIV-positive people [13,14]. Thus the current study was designed to measure the plasma fibrinogen concentration in Sudanese HIV-infected patients.

To our knowledge, this is only published research in Sudan that has studied the association between coagulation profiles in HIV-infected individuals. This existing study displayed that the mean and stander deviation of fibrinogen levels was statistically significantly elevated in HIV patients in comparison to the healthy control group (470.50 ±67.75 vs 214.75±21.25, P-value ≤0.001), these conclusions were in agreement. with a cross-sectional study carried out in England by Madden Erin, et al, who measured the fibrinogen levels amongst 1131 HIV infected participants and 281 normal healthful controls group, and finally concluded that the fibrinogen levels were significant statistically higher in a case group than those the normal health subject [15]. Fibrinogen is an important component of the coagulation system, and fluctuations in its circulating concentrations might predispose to thrombotic illnesses such as venous thromboembolism since higher plasma levels are associated with a nearly 4-fold increase in the risk of thrombosis [16].

Kuller LH et al (2008) [17], who investigate the relationship between inflammatory and coagulation biomarkers and mortality in HIV patients. They conclude that most etiological agent

of fatality was intimately associated to IL-6 and D-dimer concentrations; through elevating IL-6 and D-dimer levels, and discontinuing antiretroviral therapies (ART) may raise the chances of mortality even more. In line with a study conducted in Sudan by Himmat et al. in 2015, [18]. Likewise, of findings of Tien PC et al. (2010), who demonstrate 1183 HIV- inflamed women and men from different 16 geographically diverse and reported that elevated fibrinogen levels were associated with HIV patients [19]. All studies come inconsistent with our finding and agreed that the HIV patients had hypercoagulation status, and Various coagulation deficiencies in HIV-positive patients have been described, including decreased protein C and S, as well as an enlarged range of von Will brand factor [20, 21]. Fibrinogen is one of the most essential inflammatory biomarkers inside the clotting cascade and had been associated with mortality in the population [22]. As the function of fibrinogen inside the hemostatic process, we suggest that the elevation in fibrinogen leads to a hypercoagulable state and this might be attributed to stimulates the formation of blood thrombi [23]. Our findings revealed that fibrinogen level was significantly increased as disease progress (469.84 ±67.15, 472.74 ±87.75, and 478.47 ±61.92) in stage I, stage II, stage III respectively), P-value≤0.001. The normal hemostatic system is affected by a variety of conditions, with HIV infection being one of the most frequent causes of hemostatic dysfunction [7]. Our finding agreement with S. Karpatkin et al [24], that is because HIV infection causes substantial hemostatic complications, especially in the late stages of infection when the immune system is suppressed, and the presence of other infections or neoplasms aggravates the situation. The coagulation dysfunction observed in HIV patients (thrombocytopenia, endothelial cell dysfunction, and activation of coagulation factors) due to direct effect of the virus leading to a variety of consequences, this due to the capacity of HIV to link to the host cell's receptor, which is situated on the cell's surface. With the guidance of Glycoprotein 120, human cells with the CD4 receptor, co-receptor chemokines ligand 4 (CXCR4), and chemokines receptor 5 (CCR5) communicate with HIV (gp120). This combination lowers nitric oxide expression, resulting in endothelial cell dysfunction and reduced vascular endothelial cell immune function [25, 26].

Concerning HIV stage progressions, we revealed that the platelets count, PT, and PTT levels were significantly decreased and prolonged (P-value 0.054, 0.05, 0.041) sustainably upon disease progress, and inversely fibrinogen concentration was significantly increased. Our observation was in agreement with Seyoum M et al [27] who conclude that the platelets count was

significantly lower (0.001) in HAART-naive HIV-infected adults compared to HIV-infected adults who were using HAART. That is because HAART treatment has been noted in studies to lower viral load through increasing CD4 count and platelet production [28]. When compared to HAART-naive individuals, the drop of viral load and immunological reconstitution in HIV-infected people on HAART may lead to elevated platelet count. Furthermore, lowering viral load may help to reduce HIV-related hypercoagulative conditions. [29].

Stating the Limitation of the study:

The current study has some limitations which should be stated. First, the result of the study can't be generalized due to the small sample size, also the coagulation profile investigated regardless of HIV therapy of participants. Additionally, only the most essential coagulation parameters such as PT, APTT, and platelet count were assessed. As factor assay wasn't included, hence it was unable to determine the exact causation of prolonging PT and APTT. Furthermore, the study did not include the measurement of inflammatory markers such as interleukins encountered, finally, our participants were assessed regardless of Antiretroviral Thereby (ART), and highly active antiretroviral therapy (HAART) drugs.

Conclusion:

HIV-infected patients have significantly increased plasma fibrinogen levels than normal healthy control and exhibit a hypercoagulation state; this prone likely to be an increase the risk of thrombosis.

Data Availability: All datasets generated or analyzed during this study are included in the manuscript.

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