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~~ becoming compromised. [1]. HIV-associated thrombus formation ~~had been ascertained~~ during the HIV interval is ascertained and is well documented in the literature. [2]. (Provide additional reference) Numerous hemostatic errors ~~malformations~~ ~~in HIV patients~~ have been established in HIV patients, enabling mechanisms for hypercoagulability and an elevated chance of thrombosis. [3] (provide additional reference). Infection with HIV causes systemic inflammatory disease with prominent hematological disorders. Such abnormalities increase 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].

(Discuss arterial and venous thrombosis in added detail, in relation to coagulopathies, fibrinogen concentration, PT and PTT, especially in HIV setting)

The pathophysiology of HIV propose that ~~it~~ enhanced microbial product and migration through intestinal mucosa, as a result of persistent destruction to lymphatic tissue mucosa, ~~outcomes~~ result in the stimulation of monocyte, representation of tissue factor, and pathogenic hypercoagulability. [6]. (provide additional reference). Increased ~~activation of~~ platelets activation may ~~even~~ also 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]. (provide additional reference) Platelets have been proved in vitro to incorporate particles of HIV, and virus-infected platelets have been demonstrated to produce activating signs [10].

Aim

The present study was aimed at the measurement of fibrinogen levels and ~~other~~ related hematological parameters among Sudanese HIV patients. (Same manner as is in platelets, provide details of/discuss pathophysiology of fibrinogen level, PT and PTT providing references) (add PT and PTT in title of your article)

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~~ are followed at 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~~ healthy control group (Age and sex were matched between case and control group).

(Outline Sample Size Calculation, providing Formula and Reference Study in case you had calculated Sample Size. Outline any Sampling of subjects carried out, and detail Sampling Method – if not state, 'Convenience Sampling' is done).

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 who 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.

(Outline exact method how Thrombolyzer XRC measure fibrinogen concentration, calibration required and carried out, who carried out measurement, where and when, beside qualification of staff carrying out measurement, if not by authors)

Data collection and analysis:

The data was collected using a directly structured questionnaire (outline content of questionnaire, and indicate whether validated (Alpha Cronbach)) and analyzed by computer software SPSS Version 21. The parameters were compared in mean and ~~Stander division~~ Standard Deviation (SD) using the T-test. The P-value was set as 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 and kept very confidentially.

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 (Sample size is enough to do a valid t-test) , 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 PTT (the theory?). Furthermore, the study did not include the measurement of inflammatory markers such as interleukins (study can be valid without simultaneous interleukin measurement and analysis) encountered. Finally, our participants were assessed regardless of Antiretroviral Thereby (ART), and highly active antiretroviral therapy (HAART) drugs. (Study still valid in a situation not taking these factors in account)

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~~ healthy control was 37.1±0.91 SD, with equal gender distribution~~; no statistically significant difference reported.~~ (You selected 'controls' in such a manner). 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~~ provided 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~~ (In this analysis, you only compare mean, by also using SD, but not compare SD) 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 at ~~to~~ different HIV stage - 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

(In Table 3, you must compare between Stage 1 and Stage 2, Stage 2 and Stage 3 beside Stage 1 and Stage 3)

(In a state of hypercoagulopathy causing arterial and venous thrombosis, would you not expect increased platelet count instead of converse, and you had observed in your Introduction 'Such abnormalities increase in the last stage of disease')

Discussion:

Human immunodeficiency virus (HIV) ~~plays a pivotal part in~~ affects coagulation system activation and increases the risk of arterial and venous thrombosis. ~~that leads to atherosclerosis~~ (Arterial and venous thrombosis are separate entities from atherosclerosis, and in such could cause severe serious disease and death, frequently sudden – the worry is not the slower atherosclerotic disease) [11, 12]. For decades, hemostatic alterations in coagulation factor concentrations and state of hypercoagulation have been identified in HIV-positives ~~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 (published already?) research in Sudan that has studied the association between coagulation profiles in HIV-infected individuals. This existing study displayed that the mean ~~and stander standard deviation~~ 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~~ findings ~~were~~ are in agreement. with a cross-sectional study carried out in England by Madden Erin, et al (year?), who measured the fibrinogen levels amongst 1131 HIV infected participants and 281 ~~normal healthful~~ healthy controls ~~group~~, and ~~finally~~ concluded that the fibrinogen levels were significantly ~~statistically~~ higher in a case group than those the ~~normal~~ healthy subject [15]. Fibrinogen is an important component of the coagulation system, and ~~fluctuations~~ increase 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]. (provide additional references, and discuss in added detail)

Kuller LH et al (2008) [17], ~~who~~ investigated 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. (What is relevance of IL-6 and D-dimer to coagulation factors in HIV, and thus to your study?) - 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]. (Discuss in added detail) All studies come ~~in~~consistent 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 (discuss in detail association between 'protein C and S' in HIV and health in a relation to coagulation factors and venous/arterial thrombosis) , as well as an increased ~~enlarged~~ range of von Willebrand factor [20, 21]. Fibrinogen is one of the most essential inflammatory biomarkers inside the clotting cascade and had been associated with mortality in the (HIV?) 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~~ stimulate the formation of blood thrombi [23].

(Provide data on actual incidence of venous/arterial thrombosis, and fatality caused by thrombosis, in HIV by various Stage)

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 (year?) [24], that it 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~~ additional infections or neoplasms aggravates the situation. The coagulation dysfunction observed in HIV patients (thrombocytopenia, endothelial cell dysfunction, and activation of coagulation factors) is 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 progression, we revealed that the platelets count, PT, and PTT levels were significantly decreased and prolonged (P-value 0.054, 0.05, 0.041) (Table 2 show 0.000, 0.002 and 0.005 respectively) sustainably upon disease progress, and inversely fibrinogen concentration was significantly increased. Our observation was in agreement with Seyoum M et al (year?) [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. (In hypercoagulable states you would expect increased platelet count) 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 reduced HIV-related hypercoagulative conditions. [29].

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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(Follow ‘Authors’ Guidelines’ at this Journal’s website in a concern of style of listing of References**)**

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