Original Article

Differential Plasma Levels of D dimer and P Selectin and Their Correlation with CD4+ Cell Counts in Pretreatment HIV positive Patients

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Abstract

Background: Untreated HIV infection is associated with an increased risk of venous thromboembolism (VTE) with attendant high morbidity and mortality. Plasma levels of D-dimer and P-selectin are known markers of coagulation and platelet activation, respectively, and are potentially useful in VTE screening. The aim of this study was to assay D-dimer and P-selectin levels in pretreatment HIV-positive patients and to assess their correlation with the immune status of the patients based on CD4+ counts. **Materials and Methods:** This was a comparative cross-sectional study of 90 pretreatment HIV-positive patients and HIV-negative normal blood donors selected as a comparison group (total n = 180). The study was carried out at Aminu Kano Teaching Hospital, Kano, Nigeria. Plasma D-dimer and P-selectin levels were assayed using standard enzyme-linked immunosorbent assay protocols, whereas CD4+ counts were measured using a standard flow cytometry technique. **Results:** The mean plasma D-dimer level in the study group (320.3 ± 148.9 ng/ml) was higher than that of the comparison group (299 ± 175.2 ng/ml), but the difference was not statistically significant (P = 0.390). Similarly, the plasma P-selectin level in the study group was higher than that of the comparison group and the difference was statistically significant (P < 0.001). Both plasma D-dimer and P-selectin levels negatively correlated with CD4+ counts, but only P-selectin levels were statistically significant. **Conclusion:** Plasma P-selectin level is significantly high in pretreatment HIV patients, negatively correlated with CD4 + counts, and maybe a useful marker for immunological failure and assessment of the risk of VTE in HIV-infected patients.

Keywords: CD4+ count, D-dimer, HIV, P-selectin, thromboembolism

INTRODUCTION

HIV infection has been recognized as a prothrombotic condition and HIV-associated venous thromboembolism (VTE) is associated with high morbidity, mortality, and increased rates of recurrence.[1] Various established and novel biomarkers associated with VTE have been investigated with regard to their potential for predicting primary or recurrent VTE, thereby facilitating diagnosis and optimizing the clinical management of patients.

D-dimer is a fibrin degradation product containing two cross-linked D fragments of fibrin protein. It is formed when circulating plasmin cleaves the fibrin that is formed following activation of the coagulation cascade, either by contact with damaged blood vessel wall and exposure to collagen in the tissue space (extrinsic pathway) or by activation of factor VII by tissue activating factors (intrinsic pathway).[2] D-dimers are not normally present in human plasma, except when the coagulation system has been activated, with resultant thrombosis. [2] As D-dimer levels rise during an acute

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event of VTE, testing for D-dimer has been explored as a tool for the diagnosis of VTE and has been integrated into diagnostic algorithms in the management of patients with suspected VTE. D-dimer can be measured by an enzyme-linked immunosorbent assay (ELISA) with a reference level of <500 ng/ml. A negative D-dimer result is useful for excluding the diagnosis in patients with suspected VTE. [2]

P-selectin is a glycoprotein present on the membrane of α-granule of resting platelets that joins the plasma membrane when platelets are activated. The expression of P-selectin on circulating platelets has been used as an indicator of an *in vivo* activation of platelets. [3] P-selectin is also present in the Weibel–Palade bodies of endothelial cells and the molecule joins the plasma membrane when endothelial cells are activated. Soluble P-selectin is present in the plasma of humans. [3] The role of P-selectin in promoting hypercoagulable environment has been described in HIV-infected patients and found to be strongly and independently associated with VTE. [4] P-selectin induces the generation of microparticles from leukocytes and upregulates the expression of tissue factors on monocytes. [5] Some studies have demonstrated that patients with impending or acute VTE show elevated P-selectin levels, which have been shown to have a comparable diagnostic value to D-dimer in patients with confirmed DVT. [6] In this study, D-dimer and P-selectin were assayed in pretreatment HIV-infected patients attending Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria, and we examined the potential usefulness of these biomarkers in screening for VTE in this group of patients.

MATERIALS AND METHODS

Study design and participants recruitment

We conducted a comparative cross-sectional study of 180 participants attending AKTH consisting of 90 pretreatment HIV-positive persons and 90 HIV-negative apparently healthy blood donors selected from the Blood Donor Clinic of AKTH to serve as the comparison group. The study was carried out for 3 months from October 2016 to January 2017. Adults (age ≥18 years) and both males and females were enrolled in the study. Women who were pregnant or breastfeeding and those receiving hormonal contraception were excluded from participation.

Data collection

Basic demographic and clinical data were collected using a tructured questionnaire and a review of patients' clinical notes.

Laboratory analyses

Venous blood specimens were collected from a prominent antecubital vein using aseptic techniques. Commercial ELISA kits for D-dimer (D2D, Cloud-Clone Corp., USA) and for human P-selectin (Elabscience, USA) were used for D-dimer and P-selectin assays, respectively. Initial CD4+ counts for the HIV-positive patients were performed using the standard flow cytometry technique on CyFlow (Sysmex, Germany) and classified according to the Centers for Disease Control and Prevention (CDC) classification guidelines.

Ethics considerations

The study protocol was approved by the Research Ethics Committee of the AKTH. All participants signed informed consent before enrolment into the study.

Statistical analyses

Statistical analyses were carried out with Minitab-17 and Microsoft Excel statistical packages. Normally distributed continuous variables were described using mean and standard deviation, whereas the skewed continuous variables were reported as the median and interquartile range (IQR). Chi-square and Student's *t*-test were used to compare categorical and continuous variables, respectively. Pearson's correlation test was used to correlate plasma D-dimer and P-selectin levels with the baseline CD4+ counts. The critical level of statistical significance was set at a $P < 0.05$.

RESULTS

Participants in this study comprised 36 males and 54 females, with median age and IQR of 32 years (IQR = 19–74 years). The comparison group consisted of apparently healthy 20 HIV-negative blood donors comprising 69 males and 21 females, with a mean age of 30.0 ± 8.21 years and a median age of 30 years (IQR = 18–53 years) [Table 1].

The mean CD4 cell count was 323.8 ± 260.2. The baseline CD4+ cell counts of the participants were classified according to the CDC classification as presented in Table 1. The mean D-dimer values were 320.3 ± 148.9 ng/ml and 299.4 ± 175.2 ng/ml for HIV-positive participants and the comparison group respectively, whereas the average P-selectin values were 22.64 ± 6.04 ng/ml and 18.73 ± 5.48 ng/ml, respectively [Table 2]. Although a higher mean value of D-dimer was recorded in the study group, there was no statistically significant difference ($P = 0.390$). However, there was a statistically significant difference in the mean values of P-selectin between the study and comparison groups, $P < 0.001$. Furthermore, plasma D-dimer levels were found to be negatively correlated with CD4+ counts, but this was not statistically significant. There was a negative correlation between P-selectin values with CD4+ count and this is statistically significant [Table 3].

Table 1: Baseline characteristics of participants

	Study group (*n*)	Control group (n)	Total (*n*)
Age group (years)			
10-19	3	6	9
20-29	25	41	66
30-39	33	31	64
40-49	18	9	27
50-59	7	3	10
≥60	4	0	4
Total	90	90	180
Mean ± SD	35.73 ± 10.82	30.0 ± 8.21	32.867± 9.99
Sex			
Male	36	69	105
Female	54	21	75
Total	90	90	180
CD4 count (cells/µl)*			
<200	36	-	-
200-499	35	-	-
≥500	36	-	-
Total	90	-	-
Mean ±SD	323.8 ± 260.2	-	-

*CD4 cell counts of the patients were classified according to the CDC classification guidelines

Table 2: Comparison of the studied parameters between the study and comparison group

	Study group	Control group	*P* value
D-dimer (ng/ml)	320.3 ± 148.9	299.4 ± 175.2	0.390
P-selectin (ng/ml)	22.64 ± 6.04	18.73 ± 5.48	<0.001

Table 3: Correlation of D-dimer and P-selectin levels with CD4+ counts in the study group

Correlation	CD4+ count and D-dimer	CD4+ count and P-selectin
Pearson's r	-0.203	-0.3123
P-value	0.055	0.0027

DISCUSSION

This study assessed the differential plasma levels of D-dimer and P-selectin in pretreatment HIV-positive patients as compared to healthy controls. The demographic characteristics of participants in this study are largely similar to studies conducted in our environment. [7-10] Our findings suggest that most of the patients were immunologically stable with 47 54 (60%) having ≥200 cells/µ at baseline.

In this study, we observed a higher mean value of D-dimer in the study group than in the comparison group but the difference was not statistically significant. The difference observed could be attributed mainly to HIV infection as participants in both groups were apparently healthy at the time of recruitment. Furthermore, Baker *et al*.[11] demonstrated significantly higher levels of D-dimer before the commencement of ART and observed a decline in the plasma concentrations following treatment, suggesting that untreated HIV infection is associated with elevated D-dimer levels. Plasma D-dimer measurement is commonly used as a screening test in patients suspected of having thromboembolic events. It is, however, nonspecific as studies have demonstrated that D-dimer levels are also elevated in infections and in advanced age. [12] However, a normal D-dimer result almost always rules out thrombosis, and here lies the value of D-dimer assay in VTE screening. [12]

Our study also showed that the mean plasma P-selectin level in the study group was significantly higher in the HIV-positive patients than in the comparison group. The difference here also could be a result of the HIV infection. Mayne *et al*.[13] demonstrated that platelets from HIV-infected patients are activated and express more P-selectin than platelets from normal controls. We observed a negative correlation between plasma D-dimer levels and CD4+ cells count, although not statistically significant. This inverse relationship is expected, as lower CD4+ counts are associated with uncontrolled HIV infection and elevated D-dimer levels as previously reported.[11] Our study also showed that plasma P-selectin levels were negatively correlated with CD4 + cells count and were statistically significant. Our findings are similar to those of Servais *et al*.,[14] who showed that increased P-selectin is associated with worsening immunologic parameters in HIV-positive patients.

CONCLUSION

Our study showed significantly higher plasma P-selectin levels in pretreatment HIV-positive patients compared to healthy controls, and these levels correlated with worsening immunological status based on CD4+ counts. Hence, the P-selectin level may be a useful marker for immunological failure and can be explored as a marker for assessing the risk of VTE in HIV-positive patients.

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Conflicts of interest

There are no conflicts of interest.

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