Correlation of CD4 Count with Platelet Count, Prothrombin Time and Activated Partial Thromboplastin Time among HIV Patients in Benin City, Nigeria

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ABSTRACT

Objective: *To determine the effect of CD4 count on platelet count, prothrombin time (PT) and activated partial thromboplastin time (APTT) among HIV patients.*

Method: *Blood samples were collected from 100 subjects consisting of 70 HIV treatment naive patients and 30 HIV seronegative individuals. Platelet count, CD4 count, PT and APTT were performed on the blood samples using standard techniques.*

Result: *HIV-positive patients had significantly (*$p < 0.001$*) lower CD4 and platelet counts than HIV-negative subjects. Also, PT and APTT were significantly (*$p < 0.001$*) higher in HIV patients compared with their HIV negative counterparts. Among the HIV-infected patients, platelet count did not differ significantly (*$p > 0.05$*) between those with CD4 count < 200 cells/μL and those with CD4 count ≥ 200 cells/ μL. However, PT and APTT were significantly (*$p < 0.005$ *and* $p < 0.001$ *respectively) higher in HIV patients with CD4 count < 200 cell/μL. Only PT significantly correlated with CD4 count (*$r = 0.5406$, $p < 0.001$*) and this correlation was observed only among HIV patients with CD4 count < 200 cell/μL (*$r = 0.6227$, $p < 0.001$*).*

Conclusion: *HIV patients with CD4 count < 200 cell/μL have higher PT and APTT values; PT only correlated with CD4 count and endothelial activation is suggested as the possible mechanism for the coagulation defect.*

Correlación Entre el Conteo de CD4 y el Conteo de Plaquetas, el Tiempo de Protrombina y el Tiempo de Tromboplastina Parcial Activada entre Pacientes de VIH en la Ciudad de Benin, Nigeria

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RESUMEN

Objetivo: *Determinar el efecto de conteo de CD4 sobre el conteo de plaquetas, el tiempo de protrombina (PT) y el tiempo de tromboplastina parcial activada (TTPA) entre pacientes de VIH.*

Método: *Se recogieron muestras de sangre de 100 sujetos formados por 70 pacientes sin experiencia previa en el tratamiento contra el VIH y 30 individuos VIH seronegativos. Se realizaron conteos de plaquetas, conteos de CD4, PT y TTPA, en muestras de sangre usando técnicas estándares.*

Resultado: *Los pacientes con VIH tuvieron conteos de plaquetas y de CD4 significativamente más bajos que los sujetos VIH negativos. Asimismo, tanto el PT como el TTPA fueron significativamente más altos (*$p < 0.001$*) en pacientes con VIH, en comparación con sus contrapartes VIH negativos. Entre los pacientes infectados por VIH, el conteo de plaquetas no presentó diferencias significativas (*$p > 0.05$*) entre aquellos con conteo CD4 < 200 células/μL y aquellos con conteo CD4 ≥ 200 células/ μL. Sin embargo, el PT y el TTPA fueron significativamente más altos (*$p < 0.005$ *y* $p < 0.001$ *respectivamente) en pacientes con VIH con conteo CD4 < 200 células/μL. Solamente el PT estuvo significativamente correlacionado con el conteo CD4 (*$r = 0.5406$, $p < 0.001$*) y esta correlación fue observada sólo entre pacientes con VIH con conteo CD4 < 200 células/μL (*$r = 0.6227$, $p < 0.001$*).*

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Conclusión: *Los pacientes de VIH con conteo CD4 < 200 células/μL, poseen valores de PT y TTPA más altos; el PT mantenía correlación solamente con el conteo de CD4, y se sugiere la activación endotelial como posible mecanismo para el defecto de coagulación.*

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INTRODUCTION

A number of coagulation abnormalities have been described in human immunodeficiency virus (HIV) disease (1). High levels of plasma von Willebrand factor have been reported in HIV disease and might be indicative of activated endothelium (2). Endothelium is involved in important homeostatic mechanisms of non-thrombotic vascular surfaces, vascular tone regulation and immunomodulation (3, 4). Injured endothelium leads to localized inflammatory response of which the direct consequence is the occurrence of occlusive thrombotic events mediated between leucocyte recruitment and platelet adhesion and aggregation, blood clotting activation and fibrinolysis derangement (4, 5). HIV infection has been associated with endothelial dysfunction (6). HIV infection-associated endothelial dysfunction may therefore result in activation and consumption of coagulation factors and ultimately coagulation defect.

In HIV infection, the liver is affected. The liver is the major organ responsible for the synthesis of most coagulation factors and infection of the liver by HIV can lead to abnormal production of coagulation factors. The $CD4^+$ count is used to measure immune status and HIV disease progression (7).

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are screening tests for the extrinsic and intrinsic clotting systems respectively. They detect deficiency or inhibition of clotting factors in either system, and are the first tests in screening for coagulation disorders. As HIV infection progresses, endothelial dysfunction and liver damage will increase and this may result in severe clotting impairment. This study aims to determine the effect of $CD4^+$ count on platelet count, PT and APTT among HIV treatment naïve patients.

SUBJECTS AND METHODS

The Ethical Committee of the University of Benin Teaching Hospital, Benin City, Nigeria, approved the protocol for the study. A total of 100 subjects consisting of 70 HIV seropositive treatment naïve patients and 30 HIV seronegative apparently healthy individuals were used in this study. The HIV patients were asymptomatic, recently diagnosed and were attending HIV clinics at the University of Benin Teaching Hospital, Benin City, Nigeria. Exclusion criteria included symptoms of any disease, history of bleeding disorders and use of any anticoagulant drug within two weeks prior to specimen collection. Informed consent was obtained from every subject.

Ten millitres of blood were collected from each subject and dispensed into EDTA (5 ml) and 3.8% sodium citrate (4.5 ml of blood + 0.5 ml of sodium citrate) containers and mixed. Prothrombin and activated partial thromboplastin time were performed using the sodium citrate sample while the EDTA sample was used to determine platelet count and CD4 count. Prothrombin time (PT), activated partial thromboplastin time (APTT) and platelet count were performed by the methods described by Dacie and Lewis (8) while CD4 count was determine using flow cytometry (Partec Gmbh, Germany) following the manufacturers instructions.

The data obtained were analysed manually using unpaired *t*-test and Pearson's product moment correlation.

RESULTS

The CD4 count and some haemostatic values among the study subject are shown in Table 1. CD4 and platelet counts were significantly ($p < 0.001$) lower in HIV patients compared with their seronegative counterparts. While PT and APTT were significantly ($p < 0.001$) lower in HIV seronegative in comparison to HIV seropositive patients.

Table 1: CD4 count and some haemostatic values among the study subjects

Parameter	HIV negative subjects (n = 30)	HIV positive patients (n = 70)	*p* value
CD_4 (cells/μL)	945.63 ± 208.68	352.56 ±256.00	$p < 0.001$
Platelet count ($x10^9$/L)	224.00 ± 32.09	152.04 ± 58.94	$p < 0.001$
Prothrombin time (s)	16.50 ±1.33	19:77 ± 2.62	$p < 0.001$
Activated partial Thromboplastin time (s)	38.23 ± 3.44	43.14 ± 5.98	$p < 0.001$

Figures are in mean ± standard deviation
n = number tested.

Using a CD4 count of 200 cell/μL as cut-off, the platelet counts of HIV patients with CD4 count < 200/μL, though lower, did not differ significantly ($p > 0.05$) from those with CD4 count ≥ 200/μL (Table 2). However, PT and APTT were significantly higher ($p < 0.005$) and $p < 0.001$ respectively) in HIV patients with CD4 count < 200/μL (Table 2).

Table 2: Effect of CD4 count on haemostatic parameters of HIV patients

Parameter	HIV patients with CD_4 count < 200 cell/μL (n = 25;) ≥ 200/μL (n 245)		*p* value
Platelet count ($x10^9$/L)	135.52 ± 57.16	161.22 ± 58.52	$p > 0.05$
Prothrombin time (s)	21.52 ± 2.33	19:77 ± 2.62	$p < 0.005$
Activated partial thromboplastin time (s)	46.20 ± 7.08	41.44 ± 4.52	$p < 0.001$

Figures are in mean ± standard deviation
n = number tested.

The correlation between CD4 count and some haemostatic parameters are shown in Table 3. Only prothrombin time had a significantly negative moderate correlation with CD4 count (r = -0.5406; $p < 0.001$) and this correlation was observed only in HIV patients with CD4 count < 200/μL (r = -0.6227; $p < 0.001$).

Table 3: Correlation between CD4 count and some haemostatic parameters in HIV patients

Parameter	CD4 counts of HIV patient (cells/μL)		
	All (n = 70)	≥ 200 (n = 45)	< 200 (n = 25)
Platelet count (x10^9/L)	r = 0.1422 ($p > 0.05$)*	r = 0.012 ($p > 0.05$)	r = 0.005 ($p > 0.05$)
Prothrombin time (s)	r = 0.5406 ($p < 0.001$)	r = -0.3303 ($p < 0.05$)	r = -0.6227 ($p < 0.001$)
Activated partial Thromboplastin time (s)	r = -0.3842 ($p < 0.002$)	r = 0.2402 ($p > 0.05$)	r = -0.1527 ($p > 0.05$)

* p-values; n = number tested.

DISCUSSION

HIV infection is associated with endothelial dysfunction (6) and liver damage. Both endothelial dysfunction and liver damage can result in coagulation defects. It is therefore expected that as the HIV infection progresses, the coagulation abnormalities will increase.

The study showed a significant reduction in CD4 count in HIV seropositive patients compared with their seronegative counterparts ($p < 0.001$). The HIV attacks and destroys cells with the CD4 antigen and this explains why HIV-positive patients had lower CD4 counts than HIV-negative individuals.

Like the CD4 count, the platelet count was significantly lower in HIV seropositive patients ($p < 0.001$). Impaired thrombopoiesis and production of antiplatelet antibodies have been suggested as possible mechanisms (1). Impaired thrombopoiesis can result from infection of megakaryocytes by HIV because megakaryocytes possess CD4 and CXCR4, which are known receptors for HIV, and various megakaryocyte lines are infectable with HIV (9–11).

The PT and APTT in HIV patients were significantly higher than the values in HIV-negative individuals ($p < 0.001$). HIV infection is associated with endothelial damage, which can result in blood activation and consumption of blood clotting factors (6). Also, lupus anticoagulant (LA), anticardiolipin antibodies (aCL) [1] and liver damage are seen in HIV-infected patients. All these (damaged endothelial, LA, aCL and liver damage) can affect PT and APTT and may explain the high PT and APTT values observed among HIV patients in this study.

The CD4 count is used as a measure of immune status and disease progression (7) and values < 200 cells/μL make the patients vulnerable to opportunistic infections and other AIDS–defining conditions. Thus, on the basis of CD4 count, the studied haemostatic parameters may also indicate disease progression. However, in this study, there was no significant difference ($p > 0.05$) in the platelet count of HIV-positive patients whose CD4 count were < 200 cells/μL and those with CD4 count ≥ 200 cells/μL. It has been reported that patients with AIDS have decreased platelet production, whereas patients with early onset HIV infection are more likely to have increased peripheral destruction of platelet by antiplatelet antibodies (12). Both mechanisms result in low platelet count in HIV-positive patients with CD4 count < 200 cells/μL and those with ≥ 200 cells/μL and may explain the findings in this study. The PT ($p < 0.005$) and APTT ($p < 0.001$) of HIV-positive patients with CD4 count < 200 cells /μL were significantly higher than those of HIV patients with CD4 count ≥ 200 cells/μL. The possible explanation is that as the HIV infection progressed, which is characterized by reduction in CD4 count, endothelial activation and possibly liver damage may increase resulting in consumption of blood clotting factors and/or abnormal production of liver dependent clotting factors: resulting in increase PT and APTT.

The correlation between CD4 count, platelet count, PT and APTT showed that only PT significantly correlated negatively with the CD4 count of HIV patients ($p < 0.001$). A similar picture was seen in HIV patients with CD4 count < 200 cells/μL (r = -0.6227, $p < 0.001$). The PT detects defects in extrinsic coagulation pathway. Factor VII is the only coagulation factor defect that PT detects and cannot be detected by APTT. Baker *et al* (13) reported that tissue factor (TF) released by subendothelial cells during vascular damage, binds to circulating factor VII with formation of VIIa. The TF: VIIa complex activate X to Xa and this proceeds through the usual coagulation cascade. This indicates that endothelial activation is mainly responsible for the increased PT and APTT observed in this study. Factor VII assay and the effect of CD4 count on Factor VII concentration may confirm this. It also seems likely that PT could be used as a means of HIV disease progression in resource-poor settings where CD4 count is impossible, just as anaemia is used as a marker of disease progression (14). At this cut-off value of CD4 count (< 200 cells/μL), the corresponding PT of > 21seconds may indicate lower immunity, possible appearance of opportunistic infections

and coagulation abnormalities. However, this will require further investigation to verify.

In conclusion, PT and APTT are higher in HIV patients with CD4 count < 200 cells/μL, but only PT correlates with CD4 count and the correlation was observed only in HIV patients with CD4 count < 200 cells/μL. Endothelial activation is suggested as the cause of the coagulation defect.

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