Lupus anticoagulant in Nigerian patients living with human immunodeficiency virus/ acquired immunodeficiency syndrome

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Background and purpose: Lupus anticoagulants (LACs) are frequently found in patients with human immuno-deficiency virus (HIV). This study was designed to examine the prevalence of LACs and its significance in HIV-infected Nigerian patients.

Methods: LACs were assayed, and complete blood count and direct Coombs' test (DCT) were performed for 155 participants. Patients with other conditions known to be associated with LACs such as autoimmune disease, pregnancy, malignancies, and illegal drug use were excluded. There were 104 highly active antiretroviral therapy–naive patients with HIV and 51 HIV-negative control participants.

Results: The prevalences of LACs in HIV-infected patients and controls were 2.9% and 1.9%, respectively (p = 0.973). The majority of the patients (76%) had clinical and/or immunological acquired immunodeficiency syndrome. The mean (± standard deviation) hematocrit levels of patients (0.32 ± 0.05) were significantly lower than those of the controls (0.40 ± 0.04) [p <0.0001]. Although within the normal range, the platelet count of HIV-infected patients (180 ± 667 × 10^9/L) was significantly lower than that of the controls (213 ± 80 × 10^9/L) [p = 0.026]. None of the participants had neutropenia or DCT-positivity. There was no correlation between LAC and opportunistic illness, thrombosis, or cytopenia.

Conclusions: The prevalence of LACs was low and was not associated with opportunistic illness, thrombosis, or cytopenia.

Key words: Acquired immunodeficiency syndrome; HIV; Lupus coagulation inhibitor; Neutropenia; Thrombocy-topenia; Thrombosis

Introduction

Lupus anticoagulants (LACs) are immunoglobulins directed against phospholipid-binding proteins, and are common causes of prolongation of phospholipid-dependant clotting assays such as the activated partial thromboplastin time (aPTT) and dilute Russell's viper venom time [1]. LACs are members of a large family of acquired circulating anticoagulants [2], including anticardiolipin antibodies [ACAs] and coagulation factor inhibitors (factor VIII antibodies). LACs are related, but not identical, to ACAs.

Although first described in patients with systemic lupus erythematosus [3], LACs have been noted subsequently in association with other diseases such as myeloproliferative disorders, neoplasms, pregnancy, drug therapies, and other immune disorders, including human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), although occasionally there has been no known underlying association [4,5].

Paradoxically, LACs can be associated with clinical thrombosis [4]. Clinical interest in antiphospholipid antibodies is due to the association of LACs with arterial and venous thrombosis, and they have been found

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to be stronger risk factors than ACAs [4]. In addition to the association of LACs with thrombotic disorders, some studies have associated the occurrence of these circulating anticoagulants with peripheral neuropathy [6], pulmonary hypertension [7], endocrine disorders [8], avascular necrosis of bone [9], multiorgan failure (referred to as catastrophic antiphospholipid syndrome) [10], autoimmune hemolysis, and thrombocytopenia [11].

HIV can cause polyclonal activation of B lymphocytes [12]. The resulting polyclonal antibodies may be directed against self-antigens. Some of these antibodies may have LAC specificities and may underlie some of the clinical and laboratory features of HIV/AIDS. The purpose of this study was to examine the prevalence and significance of LACs in Nigerian patients living with HIV/AIDS.

Methods

Patients and blood collection
104 consecutive highly active antiretroviral therapy–naive patients with HIV, with or without symptoms, were enrolled in the study. Fifty one apparently healthy age- and sex-matched participants were recruited as a control group. Patients with autoimmune disorders and other conditions or drug abuse known to be associated with the development of antiphospholipid antibodies were excluded. Informed consent was obtained from each participant. The study was approved by the Research Ethics Committee of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.

A detailed medical history was taken, and a full physical examination was conducted, the findings of which were documented for all patients. Blood was obtained by venipuncture and collected into plastic tubes containing sodium citrate (ratio 9:1) and potassium ethylenediaminetetraacetic acid (EDTA) to investigate for the presence of LACs and to perform a full blood cell count, CD4+ lymphocyte count, and direct Coombs' test (DCT).

Laboratory study
After double centrifugation at 2500 g for 15 min, platelet-poor plasma was immediately assayed. The CD4+ lymphocyte count was done using the Dynal® T4 Quarant Kit (Dynal Biotech, ASA, Oslo, Norway), according to the manufacturer's instructions. Full blood count was done in EDTA-anticoagulated blood using automation (ADVIA-60 Bayer Corporation, New York, NY, USA) and DCT was performed following the standard procedure.

Detection of lupus anticoagulants
Plasma samples were evaluated for the presence of antiphospholipid antibody activity by using the following screening tests: aPTT using Hemoscann® (Química Clínica Aplicada SA, Amposta, Spain), and prothrombin time using HemoStat Thromboplastin-SI (Human Gesellschaft für Biochemica und Diagnostica GmbH, Wiesbaden, Germany). For inhibitor identification, aPTT tests were performed using 1:1 mixtures of patient and normal plasma. As a confirmatory test, kaolin clotting time (KCT) was performed following the standard procedure. The presence of LACs was diagnosed when the KCT ratio was ≥1.2.

Statistical analysis
Student's t test was used to test the significance of differences between mean values. The Statistical Package for the Social Sciences (SPSS) for Windows (Version 11.0; SPSS, Chicago, IL, USA) was used for all statistical analyses. A p value <0.05 indicated statistical significance.

Results

Clinical characteristics
There were 104 patients (65 women and 39 men; ratio 1.7:1), with a median age of 36.5 years (range, 22 to 65 years). Seventy five patients (72.1%) had clinical AIDS, 17 (16.3%) had non-AIDS symptoms, and 12 (11.5%) were asymptomatic. Among the 75 patients with clinical AIDS, 42.2% had wasting syndrome, 21.2% had oro-esophageal candidiasis, 6.7% had pulmonary tuberculosis, and 1.0% had Kaposi sarcoma.

Laboratory parameters
The mean (± standard deviation) hematocrit levels of patients (0.32 ± 0.05) were significantly lower than those of the controls (0.40 ± 0.04) [$p < 0.0001$]. Although within the normal range, the platelet count of HIV-infected patients (180 ± 667 × 10^9/L) was significantly lower than that of the controls (213 ± 80 × 10^9/L) [$p = 0.026$]. None of the participants had neutropenia or positive DCT. There was no correlation between LAC and opportunistic illness, thrombosis, or cytopenia.

Seven patients and 1 control participant had prolonged aPTT. Correction of aPTT prolongation was

Table 1. Summary of participants with prolonged kaolin clotting time (KCT) [reference value, <1.2; reference range, 60-110 sec].

Participants	KCT (% test plasma in normal plasma)					KCT ratio	Lupus anticoagulant
	0/100	20/80	50/50	80/20	100/0		
Patient 1	86.5	117	191.5	198	204	2.16	+
Patient 2	66	129	137	140	162	1.95	+
Patient 3	72	138	157	165	169	1.92	+
Control	82	118	126	134	141	1.44	+

not obtained for 3 patients or the control participant when their plasma was mixed with normal plasma at a ratio of 1:1 (Table 1). The prothrombin time of all patients and controls were within the normal range. The KCT ratios of the 4 participants whose aPTT were not corrected were greater than 1.2:1, so these participants were considered suitable for antiphospholipid antibody. Fig. 1 shows the KCT plotted against the proportion of participants' plasma in a mixture with normal plasma. Patients 1 and 3 and the control participant had a type I curve, indicating the presence of antiphospholipid antibody only, while patient 2 had a type II curve, indicating the presence of both antiphospholipid antibody and coagulation factor deficiency.

Seventy six percent of patients had immunological AIDS (CD4, <200 cells/mm^3; reference range, 450-1400 cells/mm^3); the remaining 24% had a CD4 lymphocyte count of 200 to 500/mm^3 and no patients had CD4 lymphocyte counts of >500/mm^3. Forty seven control participants (92.2%) had a CD4 lymphocyte count of >500/mm^3, while 4 (7.8%) had a count of 400 to 500/mm^3 and 2 had subnormal counts of <450/mm^3.
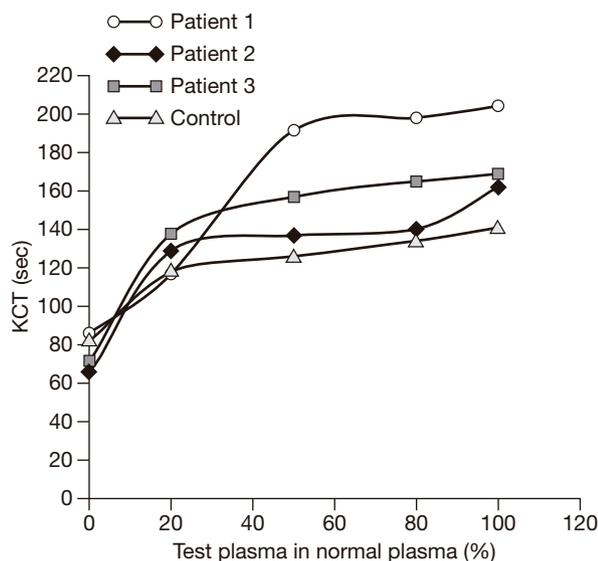
Fig. 1. Kaolin clotting time (KCT) plotted against the proportion of participants' plasma in a mixture with normal plasma.

Discussion

LAC was found in 2.9% of HIV-infected patients in this study, which was slightly higher than the controls (1.9%); the difference was not statistically significant ($p = 0.973$). Other studies have found LACs in 0% to 72% of HIV-infected patients [13-17]. The variation in prevalence may be due to clinical characteristics, the antiretroviral status of patients [13], and the type of HIV strain encountered.

The clinical features of HIV-infected patients with positive antiphospholipid antibody in this study were non-specific and not exclusive. Cough and generalized body itching were present in 2 of the 3 antiphospholipid antibody–positive patients (66.7%), and fever (n = 1), fatigue (n = 1), anorexia (n = 1), and generalized maculopapular rashes (n = 1) were also present. None of the patients had thrombosis.

As LAC is heterogeneous, several points need consideration when evaluating its activity [18-20]. The test (KCT) selected for confirmation of antiphospholipid antibody in this study has been shown to be very sensitive and specific [21,22]. The presence of antiphospholipid antibody in these patients was not associated with the presence of AIDS-defining illness, as expected. However, some studies have shown an association between the occurrence of LACs and the presence of opportunistic disorders in patients with AIDS [23]. It is possible that the lower prevalence rate in this study can be explained by the fact that the risks for these opportunistic illnesses in the local environment may be much lower than in developed countries. For example, *Pneumocystis jirovecii (Pneumocystis carinii)* pneumonia, which is commonly associated with LAC-positivity accounts for 60% of AIDS-defining diagnoses in patients in Europe and North America, but is much less common in sub-Saharan Africa, where its prevalence is consistently estimated at ≤10% among patients with AIDS [24,25]. More-over, the prevalence of these opportunistic illnesses may be decreasing globally because of improved care for patients with HIV/AIDS.

In some studies, LAC has been associated with anemia, thrombocytopenia, or neutropenia [12]. In this study, there was a significant difference in mean hematocrit values between the patients and the controls (r=10.03, $p < 0.0001$). Similarly, although the platelet count of HIV-infected patients was within the normal range ($180 \pm 667 \times 10^9$/L), this was significantly lower than that of the control participants ($213 \pm 80 \times 10^9$/L) [$p = 0.026$]. The absolute neutrophil counts for both patients and controls were within normal limits. For all of the HIV-infected patients, no correlation between LAC and cytopenias (anemia, thrombocytopenia, or neutropenia) was found. This finding is in agreement with those of other studies [14,16]. The mechanisms of anemia and thrombocytopenia appear to be multi-factorial.

These results demonstrate a low prevalence rate of antiphospholipid antibody in HIV-infected patients in Nigeria, and its presence was not associated with opportunistic illness, thrombosis, anemia, or thrombocytopenia. This study comprised a relatively small number of participants; therefore, a multicentre study is required.

Acknowledgments

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